J. Anat. (1991), **175**, pp. 105–129
With 9 figures
Printed in Great Britain

Central projections of the nodose ganglion and the origin of vagal efferents in the lamb

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(*Accepted* 10 *September* 1990)

INTRODUCTION

During the past two decades the sheep, its lamb and fetus have become an important experimental model in fetal physiology in general and in developmental respiratory physiology in particular (Jansen & Chernick, 1983; Johnston & Gluckman, 1986). The sheep is also a valuable model for the investigation of the peripheral and central neural control of swallowing (Jean & Car, 1979; Jean, Amri & Calas, 1983; Jean, 1984*a*, *b*; Amri, Car & Jean, 1984; Roman, 1986; Car & Amri, 1987; Amri & Car, 1988) and, as a ruminant, potentially for the study of gastric sensorimotor control (Harding & Leek, 1973).

These apparently diverse studies have as their common neural focus the major viscerosensory nucleus in the medulla, the nucleus of the solitary tract (nTS), which receives primary afferents from the cervical, thoracic and abdominal organs via the vagus (X) and glossopharyngeal (IX) nerves. Although these inputs have been extensively investigated with a variety of techniques in a wide range of vertebrate species (e.g. rat: Contreras, Beckstead & Norgren, 1982; Kalia & Sullivan, 1982; Leslie, Gwyn & Hopkins, 1982; Hamilton & Norgren, 1984; Norgren & Smith, 1988; hamster: Miceli & Malsbury, 1985; opossum: Culberson & Kimmel, 1972; cat: Kalia & Mesulam, 1980*a*, *b*; Kalia & Welles, 1980; Ciriello, Hyrcyshyn & Calaresu, 1981; Nomura & Mizuno, 1983; monkey: Gwyn, Leslie & Hopkins, 1985; Hamilton, Pritchard & Norgren, 1987; ferret: Fitzakerly & Lucier, 1988; dog: Chernicky, Barnes, Ferrario & Conomy, 1984; catfish: Kanwal & Caprio, 1987; skate: Barry, 1987; frog: Rubinson & Friedman, 1977; lizard: Barbas-Henry & Lohman, 1984; mallard: Dubbeldam, Brus, Menken & Zeilstra, 1979; hen: Norman & Bower, 1982; pigeon: Katz & Karten, 1979, 1983; cockatoo: Wild, 1981), there is limited anatomical information on IX and X projections in ruminants generally or the sheep in particular (Sweazey & Bradley, 1986). We have sought to remedy this situation by charting the total sensory projections of the vagus nerve in a series of lambs by injecting the nerve or nodose ganglion with either wheat germ or cholera toxin B subunit conjugates of horseradish peroxidase (WGA-HRP or CTB-HRP). Since these injections also produced extensive retrograde labelling within the medulla, a reasonably complete picture of the efferent origins as well as the afferent terminations of the vagus was provided.

MATERIALS AND METHODS

Twenty-six Romney cross lambs aged between 4 days and six weeks served as subjects. Each was anaesthetised by an intravenous injection of sodium pento-

barbitone, intubated, and anaesthesia maintained with nitrous oxide. A ventral vertical incision was made on either the left (n = 17) or right (n = 7) side of the upper neck and, using blunt dissection, the vagus nerve was located and freed from its attachment to the carotid artery and surrounding tissue over a variable distance, but never less than 2 cm. In 4 cases the cervical vagus was then cut and its proximal end bathed in a mixture of 20–30 % horseradish peroxidase (HRP: Boehringer-Mannheim Grade I) and 5·0 % wheat germ agglutinin conjugated to HRP (WGA-HRP: Sigma) in Tris buffer, pH 8·6, for 0·5–1·5 hours. Leakage of tracer from the site of injection was controlled by placing the cut nerve in a plastic trough surrounded by gelfoam swabs which were frequently replenished. In a further 4 cases the intact nerve was multiply injected via a glass micropipette (outside diameter 20–30 μm) glued to a 5 μl Hamilton syringe with 10–15 μl of either 5 % WGA-HRP (n = 2), WGA-HRP mixed with cholera toxin B subunit conjugated to HRP (CTB-HRP: McIlhinney, Bacon & Smith, 1988; 2 % in phosphate buffer) (n = 1) or CTB-HRP alone (n = 1). Leakage was controlled in a similar manner. In a further 12 cases the dissection was continued proximally until the nodose ganglion was visualised; this was then freed as far as possible from surrounding tissue. This variably involved in different cases the removal of glandular tissue, severance of the hypoglossal nerve (which was cauterised at its proximal cut end), tying off the lingual artery, and section and removal of the posterior diagastric muscle. The ganglion was then multiply injected via a glass micropipette glued to a 5 μl Hamilton syringe with 10–15 μl of either 5 % WGA-HRP (n = 9), a mixture of WGA-HRP and CTB-HRP (n = 1), or CTB-HRP alone (n = 2). In a further 2 cases the superior laryngeal nerve was injected with 5 μl of CTB-HRP.

In all these cases the tissues were thoroughly cleaned with saline-soaked swabs to remove any tracer which might have leaked from the injection, but in order to assess whether inadvertent uptake from surrounding tissues had taken place, two control injections were made. In one case the vagus was severed proximal to the nodose ganglion immediately following its injection with WGA-HRP, and in another 20 μl of WGA-HRP were 'injected' everywhere around the intact ganglion but not into it.

All but one of the lambs survived surgery and were returned to their mothers for 2–4 days. They were then deeply anaesthetised and perfused with one litre of normal saline, followed by one litre of 2·5 % glutaraldehyde in phosphate buffer, pH 7·4, and finally one litre of 10 % sucrose in phosphate buffer. Perfusion success in terms of removal of blood from the brain and good fixation was variable, but was best after bilateral carotid catheterisation preceded by an intra-arterial injection of heparin and 2 ml 2 % xylocaine as a smooth muscle relaxant.

The brains and cervical spinal cords were removed and the brains blocked either in the transverse or horizontal plane. Fifty-micrometre frozen sections were then collected serially in two series from each brain throughout the rhombencephalon and upper cervical spinal cord (C1–C2). In three cases every third section was also collected from C3–C6. All sections were treated with tetramethyl benzidine (TMB) (Mesulam, 1978) and one series was subsequently counterstained with neutral red. Both light and darkfield optics were used to view the projections which were charted with the aid of an overhead projector and drawing tube.

The formalin-fixed brains of three further lambs were cut at 50 μm and counterstained with thionine to form a normal series in the transverse, horizontal and sagittal planes.

RESULTS

The vagal complex in normal material

It is not the intention here to provide a detailed account of the cytoarchitecture of the nucleus of the solitary tract (nTS) in the lamb (Störmer & Goller, 1988), but rather to mention only those general features of subnuclear organisation germane to an interpretation of the pattern of termination of vagal afferents. In any case the general appearance of nTS in the lamb closely resembles that in the cat as described by Kalia & Mesulam (1980*a*). Thus, in the lamb, nTS has a rostrocaudal extent of approximately 8·5 mm from the spinomedullary junction to the level of the caudal pole of the facial nucleus. It has a narrow rostral pole but a large triangular cross-section at levels straddling the obex, where most of the subnuclei are in evidence in Nissl-stained material. At the base of the triangle adjacent to the area postrema lies the subnucleus gelatinosus (sg) and, ventral to this, the large medial subnucleus (mnTS). Ventrally again, and medially adjacent to the solitary tract (TS) at levels rostral to the obex, is the distinctive, circular, central subnucleus (cnTS: Altschuler *et al.* 1989) which, in horizontal section, takes the form of an ellipse. The solitary tract lies in the ventrolateral part of the solitary complex, has an interstitial subnucleus (inTS) within its substance, and is surrounded by dorsal (dnTS), dorsolateral (dlnTS), ventrolateral (vlnTS) and ventral (vnTS) subnuclei. Caudal to the obex the commissural nucleus joins the nTS of both sides dorsal to the central canal. The locations of most of the subnuclei are indicated in Figure 3.

The dorsal motor nucleus of the vagus (DMN X) does not extend as far rostrally as does nTS; its most rostral neurons are approximately at the level of the more rostral vagal afferent fascicles. The dorsal motor nucleus is medial to nTS in front of the obex but ventral to it behind. The ventrolateral border of the nucleus is indistinct at rostral levels where some vagal efferent neurons extend into dorsomedial regions of the reticular formation, in which the nucleus ambiguus is embedded ventrolaterally.

At upper cervical spinal cord levels the remnants of DMN X do not become gradually reduced but appear as relatively isolated groups of neurons interrupted in the rostrocaudal direction. This is also the case for neurons which replace the nucleus ambiguus caudally, i.e. the nucleus retroambiguus (nRA); they also form relatively isolated groups and are situated in the lateral part of the intermediate grey. The morphology of these neurons, however, becomes less like that of ambiguus neurons, i.e. less multipolar and more fusiform with their long axes orientated mediolaterally. The most caudal of the spinal DMN X groups can be seen to be in mediolateral continuity with groups of nRA where they appear in the same section, but nRA extends further caudally than DMN X. At C2 the nRA groups lie ventromedial to the lateral cervical nucleus.

Vagal projections

In only two of the four cases in which the cut cervical vagus was exposed to tracer, and in only two of the four in which the cervical vagus was injected with tracer, was there any detectable transganglionic transport to the brain and what label there was was sparse. Furthermore, the degree of retrograde labelling of vagal efferent neurons in these cases was variable, with near-total labelling of DMN X and the nucleus ambiguus in two cases, incomplete labelling of these nuclei in three others, and poor or minimal labelling in the remainder. These variable results were the reason why most cases received injections into the nodose ganglion. Such injections, particularly of CTB-HRP, were much more successful in revealing the pattern of vagal afferent

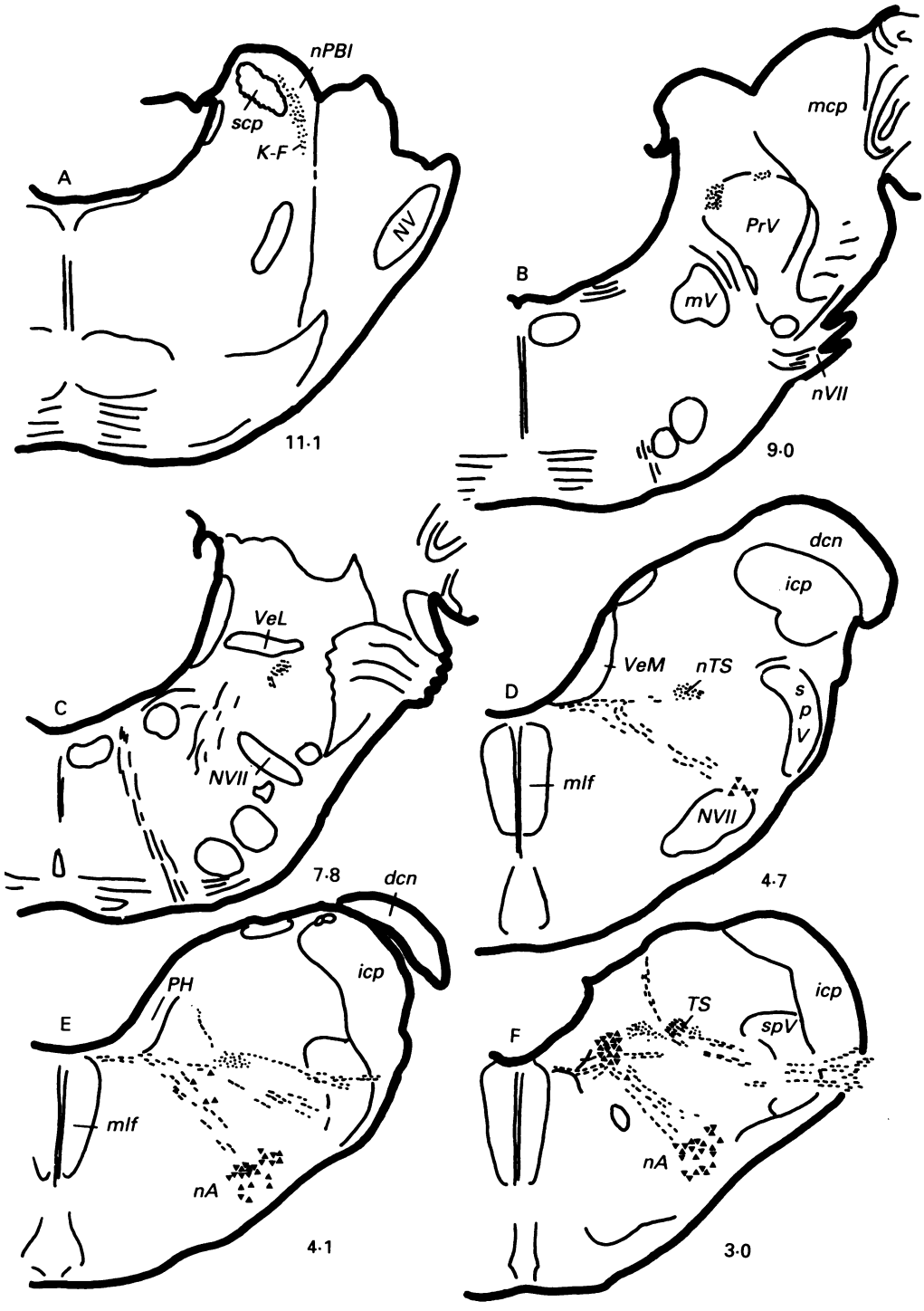

Fig. 1 (A–F). For legend see p. 110.

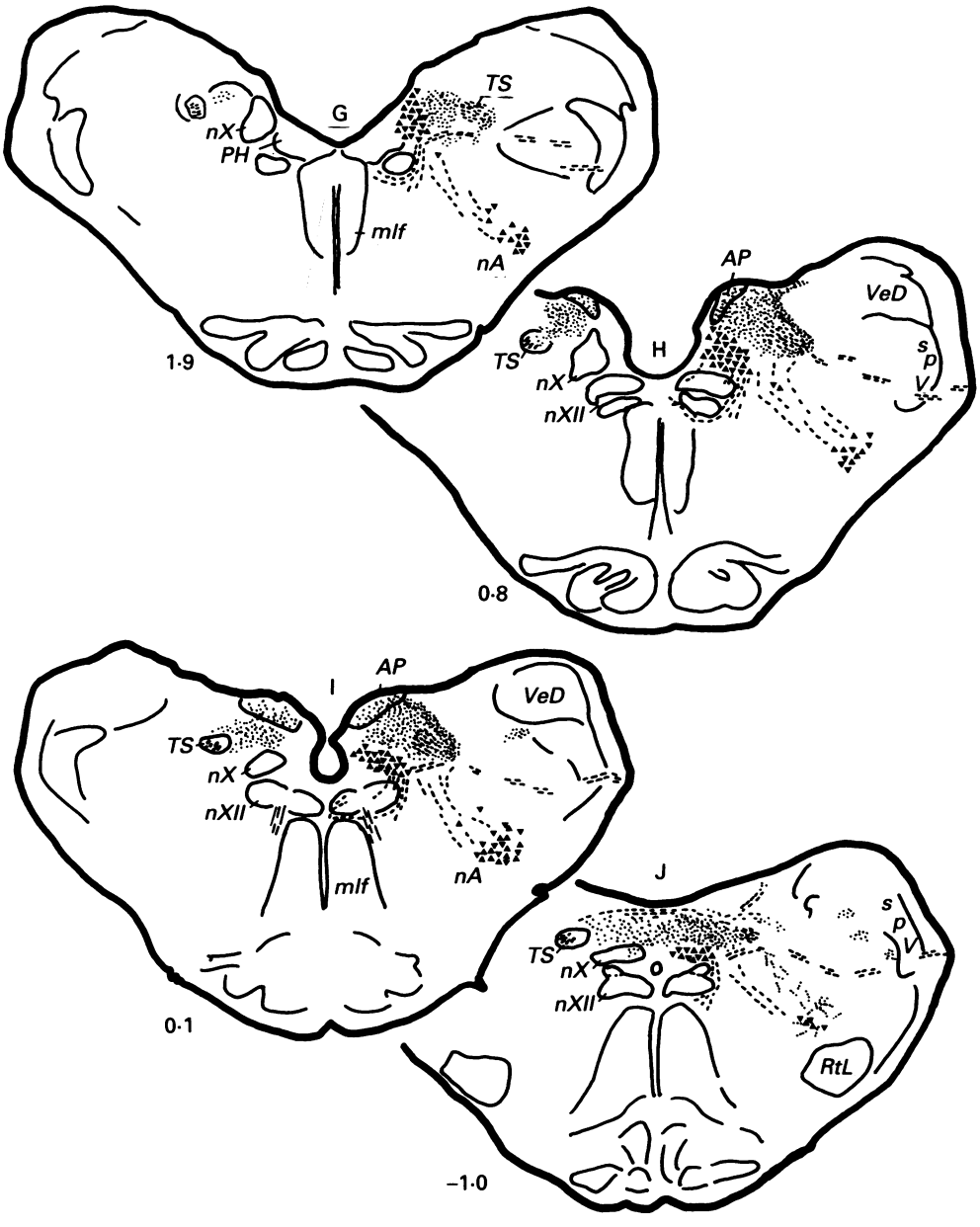

Fig. 1 (G–J). For legend see p. 110.

terminations within the brainstem, and in retrogradely labelling vagal efferent neurons.

(1) *Afferent projections*

Figure 1 presents a rostrocaudal series of schematic transverse sections depicting the central projections of the nodose ganglion in a case receiving a 15 μl CTB-HRP injection; Figure 2 presents a dorsoventral series of horizontal sections in another case receiving a similarly sized injection of CTB-HRP and WGA-HRP. All the results

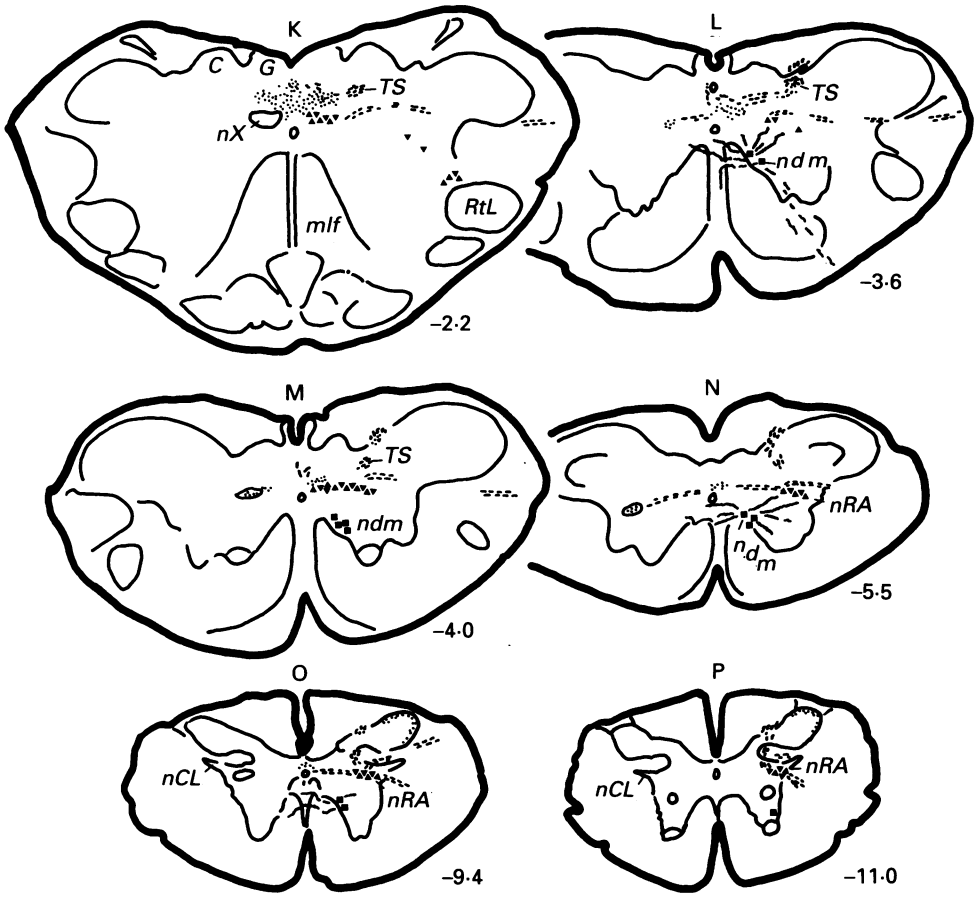

Fig. 1 (A–P). A rostral to caudal series of schematic transverse sections of the lamb brain showing the distribution of labelled fibres (dashed lines), terminals (fine dots) and cell bodies (triangles and squares) following an injection of CTB-HRP into the nodose ganglion. See text for details. Abbreviations for all Figures are given on p. 129.

depicted and to be described were confirmed to a greater or lesser extent in all other cases of ganglionic injection, whether of CTB-HRP or WGA-HRP. Selected photomicrographs of the afferent projections are presented in subsequent Figures.

(*a*) *The solitary tract* (*TS*). Labelled afferent fibre fascicles entered the lateral medulla and pierced the dorsal part of the spinal trigeminal tract and nucleus as far rostrally as the caudal pole of the dorsal cochlear nucleus, some 4·5 mm rostral to the obex (defined as the caudal point of closure of the fourth ventricle) (Figs. 1D–F, 2G, H). These fascicles could be recognised as afferent, as distinct from vagal efferent fibres, by their generally more dorsal position and their continuity with, and termination within, nTS. Vagal efferent fibres from DMN X formed separate fascicles which left the nucleus immediately ventral to nTS and left the brainstem, together with axons derived from neurons of the nucleus ambiguus, ventral and caudal to the vagal afferents (Figs. 1H–J, 2J, K).

The majority of entering fibres turned caudally to form the descending solitary tract (DTS) which coursed throughout the remainder of the medulla in the lateral part of the solitary complex of nuclei (Figs. 1–4). At the caudal end of nTS the DTS shifted

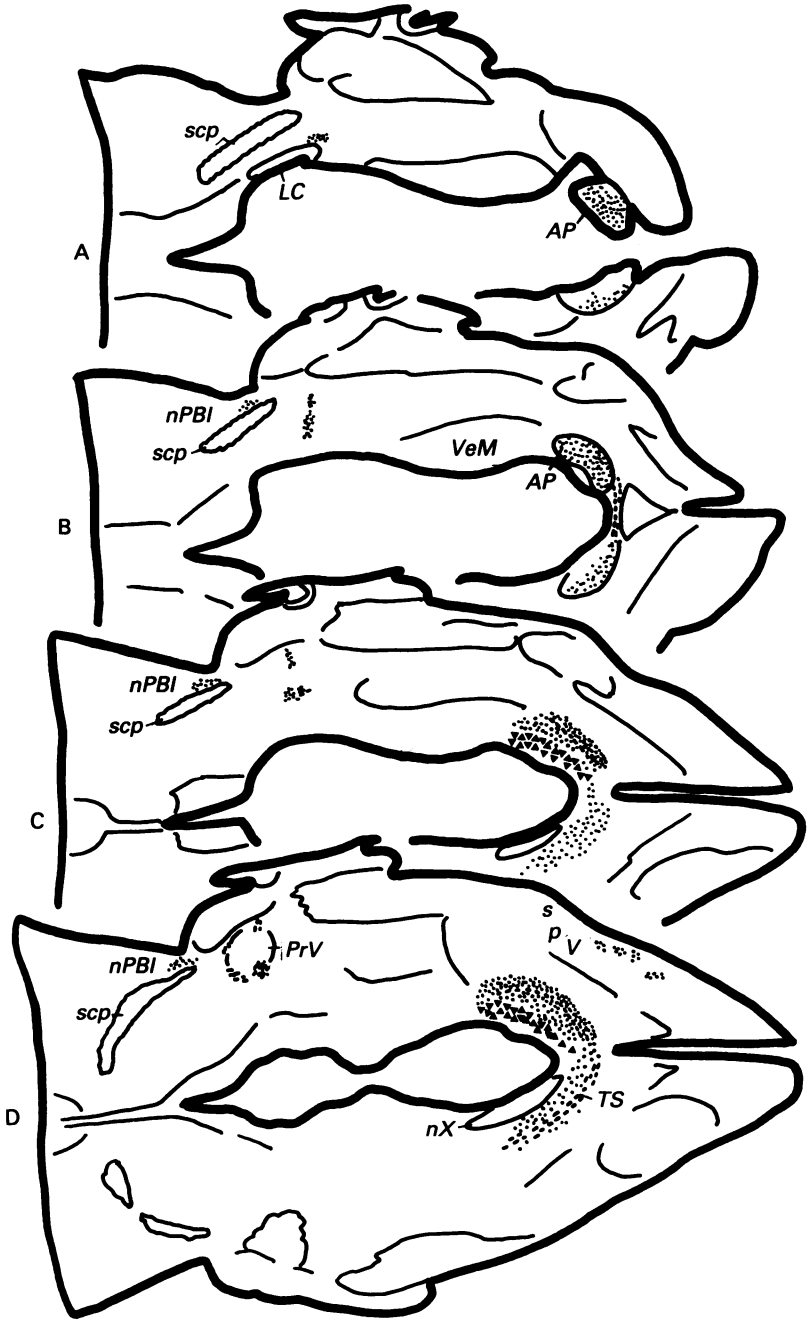

Fig. 2 (A–D). For legend see p. 113.

laterally to lie ventrolateral to the caudal end of the cuneate nucleus at upper C1 levels, and then at the medial edge of the reticular part of the base of the dorsal horn for the remainder of C1 and C2 (Fig. 1 L–P). Caudal to the obex many fibres from DTS crossed to the opposite side in the commissure of the area postrema and dorsal to the commissural nucleus of nTS (Fig. 1 J). These then ascended in the contralateral TS for approximately 2 mm.

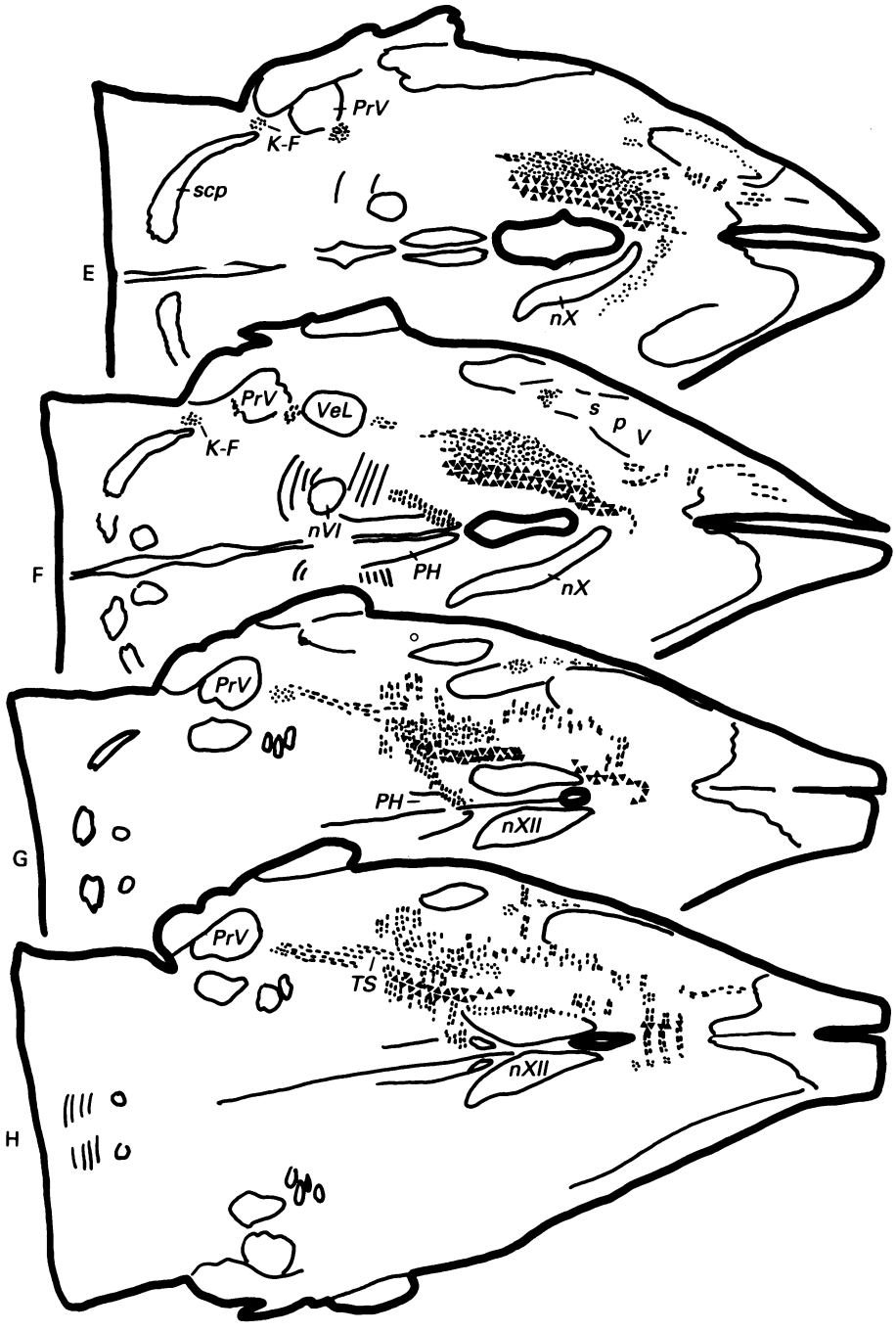

Fig. 2 (E–H). For legend see p. 113.

At levels 3·0–4·1 mm rostral to the obex a fine fascicle of labelled fibres left DTS to head dorsally towards the floor of the fourth ventricle (Fig. 1 E, F). Its final destination could not be determined.

A substantial contingent of entering vagal fibres turned rostrally from their most rostral point of entry to the medulla and coursed some 5·5 mm as an ascending

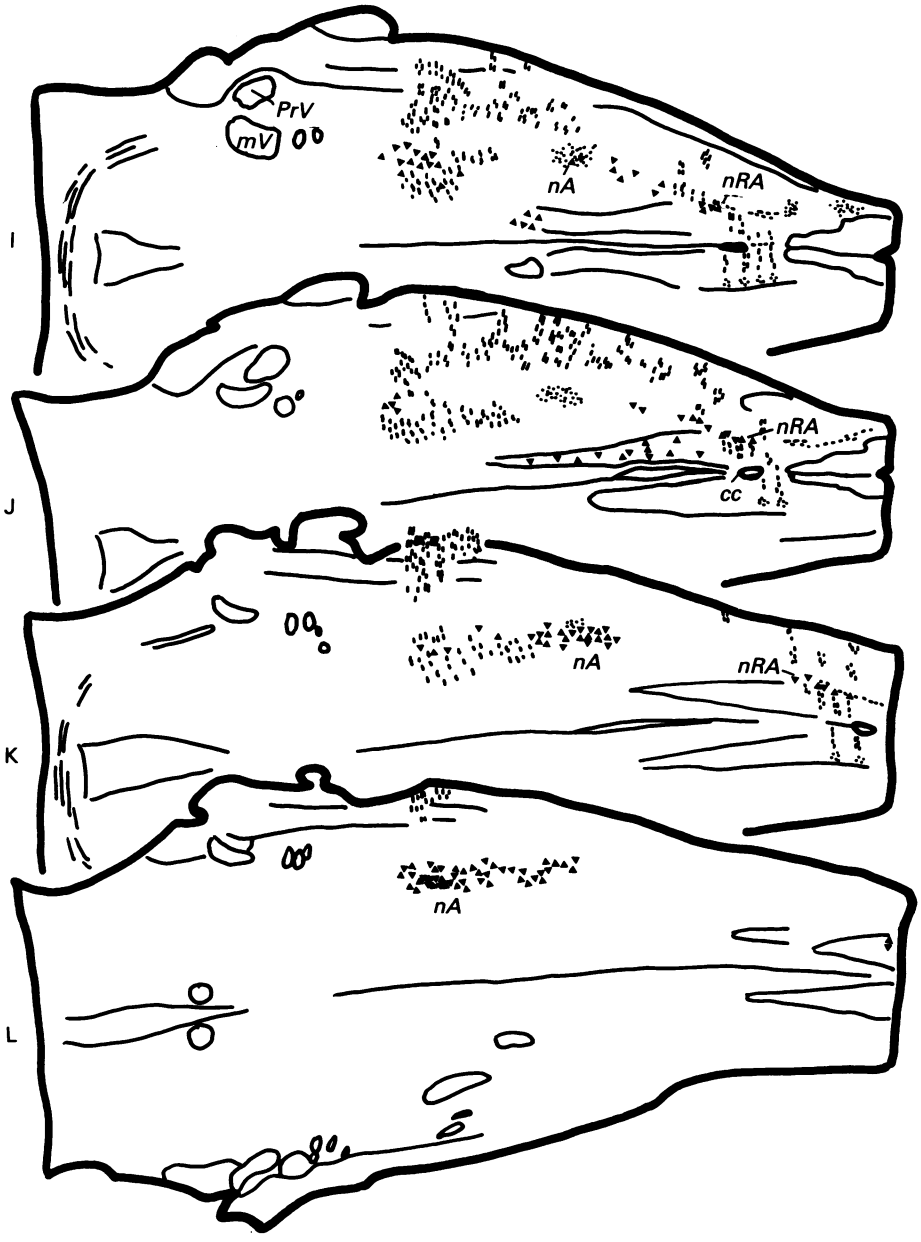

Fig. 2 (A–L). A dorsal to ventral series of schematic horizontal sections of the lamb brain showing the distribution of labelled fibres (dashed lines), terminals (fine dots) and cell bodies (triangles) following an injection of CTB-HRP and WGA-HRP into the nodose ganglion.

component of TS (ATS) into the pons (Figs. 1A–C, 2F–H, 4C). For most of its rostral course this tract was situated just medial to the dorsomedial aspect of the nucleus of the descending trigeminal tract, and, at the level of the eighth nerve root, ventral to the lateral vestibular nucleus.

(*b*) *Terminal zones of the descending solitary tract* (*DTS*). Entering vagal afferent fibres

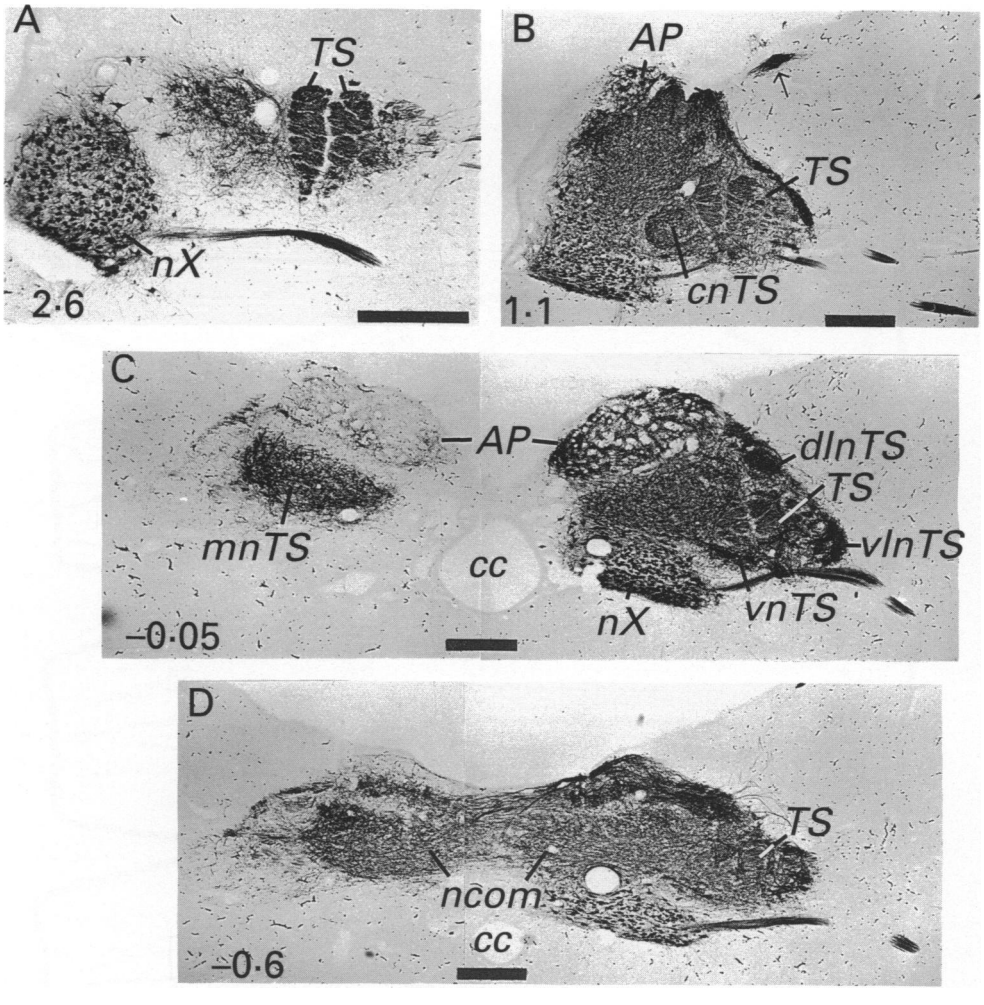

Fig. 3 (A–D). Bright-field photomicrographs showing, in the transverse plane, ipsilateral fibre and terminal labelling in *TS* and *nTS* at two levels rostral to the obex (2·6 and 1·1 mm), and bilateral labelling at two levels caudal to the obex (−0·05 and −0·6 mm) following an injection of CTB-HRP into the nodose ganglion. The arrow in B points to an unidentified patch of terminations dorsolateral to *nTS*. The dorsal motor nucleus of the vagus (*nX*) is retrogradely labelled throughout. Bars, 0·5 mm.

and the DTS terminated throughout the area postrema (AP) (Figs. 1H, I, 2A, B, 7C), and the nTS coincident with the extent of DTS (Figs. 1–4). There appeared to be no nTS subnucleus which did not receive a heavy concentration of terminations, although it was consistently found that the peripherally located subnuclei (dnTS, dlnTS, vlnTS, vnTS) were more heavily labelled than the more medially situated subnuclei (sg, mnTS, cnTS) (Fig. 3). There were also extensive terminations in the contralateral AP (reached via the commissure of AP) and in parts of the contralateral nTS rostral to the obex which were reached via fibres ascending in the contralateral TS. However, these contralateral terminations were largely restricted to mnTS, with sparse terminations in cnTS, and there was a virtual absence of them in the peripheral subnuclei (Fig. 3C). Caudal to the obex there was heavy terminal labelling within the commissural nucleus on both sides of the midline, but this was more pronounced ipsilaterally (Fig. 3D).

This caudal terminal labelling extended into upper cervical spinal levels where it lay dorsolateral to the central canal (Fig. 1 L–O). It was supplied by DTS fibres travelling through the medial part of the base of the dorsal horn (see above: (*a*) The solitary tract).

(*c*) *Terminal zones of the ascending solitary tract* (*ATS*). Terminations were apparent in the rostral part of nTS, rostral to the most rostral entering vagal fascicles (Fig. 1 D). Within the pons, a dense, ovoid patch of terminations was evident at the caudal pole of the principal sensory trigeminal nucleus (PrV), and extended rostro-dorsomedially within it, finally to lie adjacent to the locus coeruleus (Figs. 1 B, 2 A–E, 5 A). Lateral to this dorsomedial terminal zone there was a second, smaller zone of termination which capped the dorsal border of PrV (Figs. 1 B, 2 C, 5 B). Some 2 mm rostral to these terminations within PrV, labelled fibres climbed dorsally up the lateral border of the pons and terminated within a region ventrolateral and lateral to the superior cerebellar peduncle, i.e. in the Kölliker–Fuse and lateral parabrachial nuclei (Figs. 1 A, 2 B–F, 6).

(*d*) *Other terminal zones of vagal afferents.* (*i*) *The nucleus of the spinal trigeminal tract* (*nspV*). After entering the brainstem some vagal afferent fibres turned caudally within spV and terminated in dense patches primarily within the interpolated nucleus (Figs. 1, 2, 7 B). At the spinomedullary junction, labelled fibres within spV lay between the lateral margin of the caudal pole of the cuneate nucleus and the base of the dorsal horn, and at upper cervical levels these fibres terminated within both medial and lateral regions of lamina I (Fig. 1 O, P). (*ii*) *Dorsal motor nucleus of the vagus* (*DMN X*). Because of the heavy retrograde labelling of DMN X neurons ipsilaterally, it was not possible to tell whether vagal afferents terminated there; but in the contralateral DMN X where there were no labelled neurons there was some evidence of afferent terminations. Most of these were observed in the medial part of caudal regions of DMN X (Fig. 1 J), and it was difficult to distinguish them from contralaterally directed dendrites of ipsilaterally labelled DMN X neurons. (*iii*) *Ventrolateral medulla.* At the same rostrocaudal levels at which terminations were found within (the contralateral) DMN X, beaded strings of presumed terminations were also observed scattered between, and dorsal to, retrogradely labelled neurons of the nucleus ambiguus (Figs. 1 J, 2 I–K, 8 B). These terminations appeared to be supplied by vagal afferent fibres descending ventrolaterally from DTS in a position lateral to the dorsomedially directed axons of ambiguus motoneurons.

(*e*) *Terminal zones of the superior laryngeal nerve* (*sln*). The distribution of sln afferents within nTS was rostrocaudally extensive, as described by Sweazey & Bradley (1986). At levels rostral to the entering fibre fascicles, terminations were observed in the medial nTS but further caudally, about 1 mm rostral to the obex, increasingly dense terminations were present within and around the solitary tract, while the less dense terminations in the medial nTS formed a rim around the central subnucleus. Caudal to the obex, heavy terminations were present in the interstitial subnucleus (Fig. 8 D), and ventral and ventrolateral to TS. Dense patches of terminations were observed within the interpolated nucleus of spV (Fig. 8 C), and light terminations were observed within Lamina I of the upper cervical dorsal horn.

Some of the entering sln afferent fibres turned rostrally to form part of the ATS. These provided terminations to the rostral nTS, as described above, and to the dorsomedial subnucleus of PrV mentioned above in connection with the total vagal afferent projections.

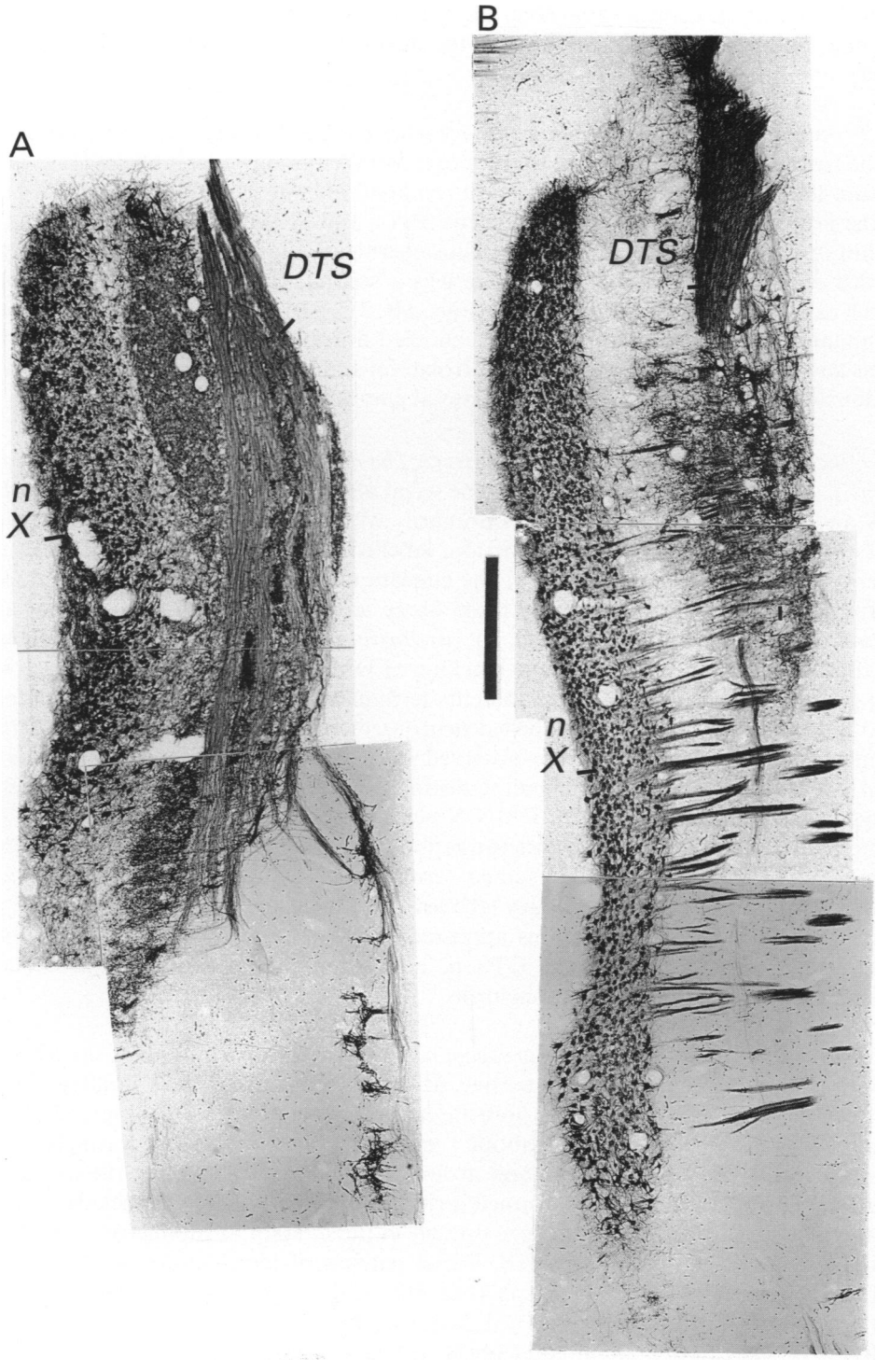

Fig. 4. For legend see opposite page.

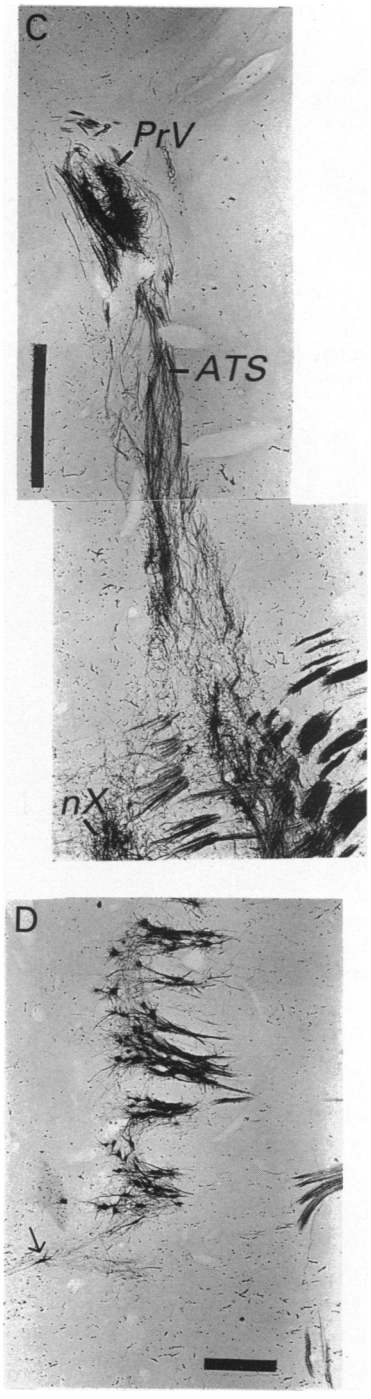

Fig. 4 (A–D). Bright-field photomicrographs showing, in the horizontal plane (rostral is up, lateral is to the right), fibre and terminal labelling in *nTS*, and retrogradely labelled neurons in DMN X (*nX*) and their efferent fascicles, following an injection of CTB-HRP and WGA-HRP into the nodose ganglion. (A) is dorsal to (B). (C) is rostral to (B), and shows the entering vagal afferents and the longitudinally extensive, ascending solitary tract (*ATS*) terminating within *PrV*. (D) is caudal to (B), and shows quasi-segmental groups of caudal DMN X neurons, one of which is displaced across the midline (arrow; see also Fig. 2G). Bars for (A), (B) and (C), 1 mm; for (D), 0·5 mm.

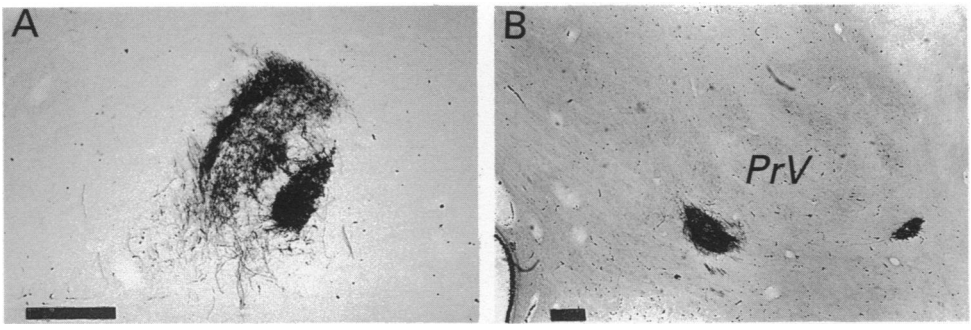

Fig. 5 (A–B). Bright-field photomicrographs showing terminal labelling within *PrV*; (A) is a transverse section, (B) is a horizontal section. Bars, 0·25 mm.

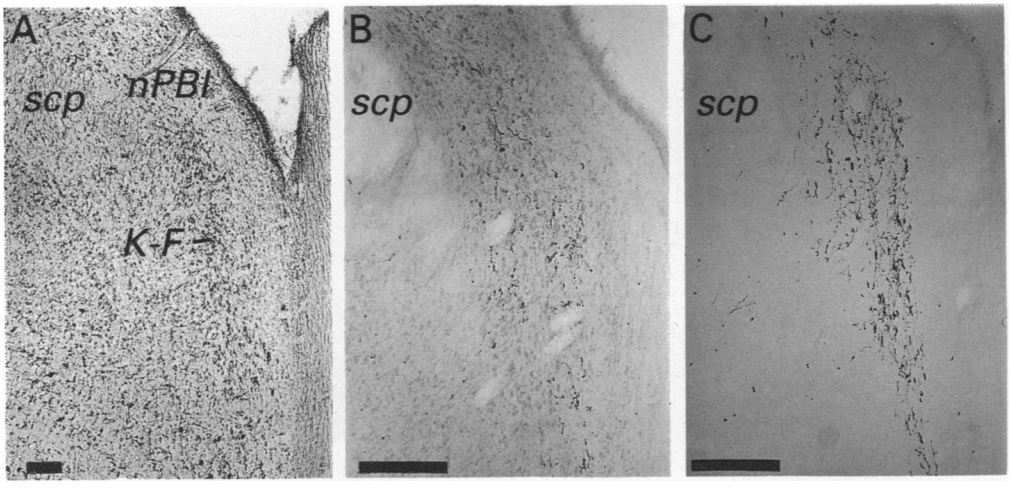

Fig. 6 (A–C). Bright-field photomicrographs showing, in the transverse plane, terminal labelling within the lateral parabrachial and Kölliker–Fuse nuclei. (A) Nissl stained section of normal brain. (B and C) Lightly counterstained and uncounterstained sections of experimental brain. Bars, 0·25 mm.

(2) *Origin of the efferent projections*

Figures 1 and 2 depict the location of retrogradely labelled neurons in the two best cases with injections into the nodose ganglion.

(*a*) *Dorsal motor nucleus of the vagus* (*DMN X*). Almost every neuron in the ipsilateral DMN X was labelled in each case (Figs. 3, 4) and, in horizontally cut cases, an occasional labelled neuron was found just across the midline (i.e. contralaterally) at caudal medullary levels (Figs 2G, 4D). The axons of DMN X neurons were seen to form discrete fascicles as they left through the medulla (Fig. 4B). As Figures 2H and 4D show, caudal DMN X neurons were found close to the midline in discontinuous clusters, reinforcing the impression of a quasi-segmental arrangement. Their axons left the upper cervical cord through the dorsolateral funiculus.

Associated with the DMN X labelling from about the level of the obex to 3 mm rostrally was a group of labelled fibres which appeared to leave the nucleus ventromedially or ventrally and to extend medially under the hypoglossal nucleus (Figs. 1F–J, 7A). Neither the origin nor the destination of these fibres could be

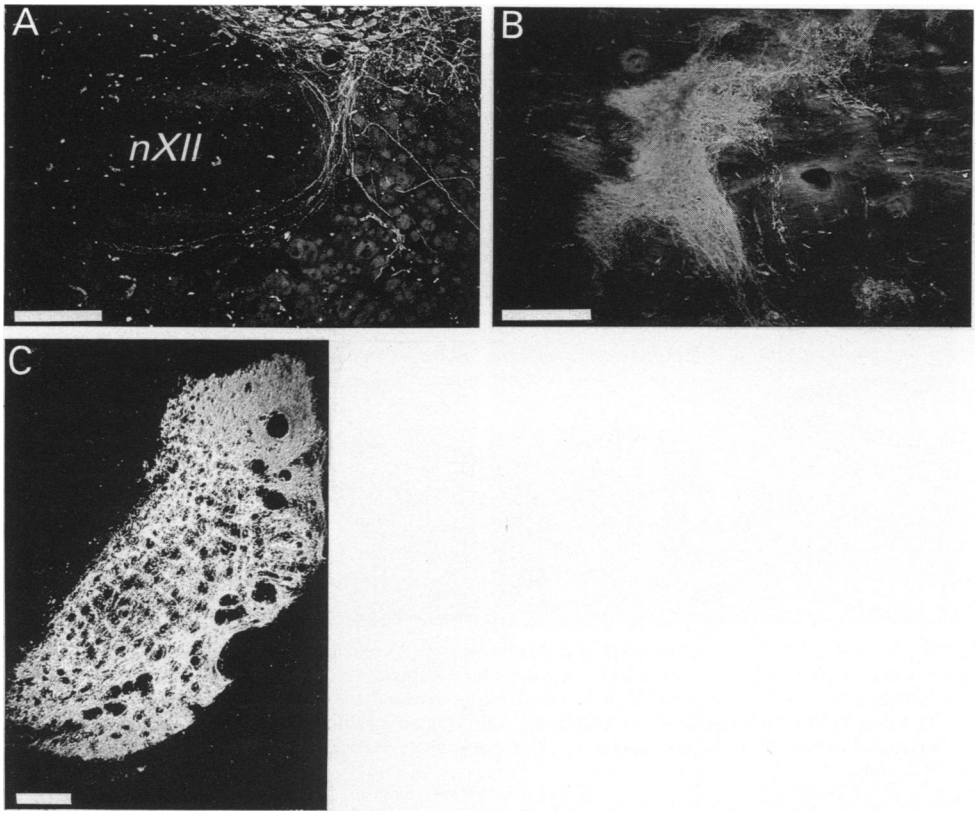

Fig. 7 (A–C). Dark-field photomicrographs depicting in (A) labelled fibres extending ventrally from the region of DMN X neurons, and ventromedial to *nXII* (transverse); in (B) a dense patch of terminations within the interpolated subnucleus of *spV* (horizontal); and in (C) terminal labelling within the area postrema (horizontal). Bars, 0·25 mm.

determined with certainty; they appeared to issue from DMN X, but they could be vagal afferents. A few of them were seen to enter the hypoglossal nucleus from below and apparently terminate there.

(*b*) *Nucleus ambiguus* (*nA*). Labelled ambiguus neurons extended from the level of the caudal pole of the facial nucleus rostrally, to the level of the lateral reticular nucleus caudally (Fig. 1). The greatest collection of these large, multipolar neurons, however, lay rostral to the obex where they formed a more medial, compact cluster surrounded laterally and ventrally by a more scattered aggregate of neurons (Fig. 8 A). The dendrites of ambiguus neurons were extensively labelled and formed a dense feltwork of fibres within and around the nucleus. Some of them reached the ventral surface of the medulla.

The axons of ambiguus neurons left the nucleus dorsomedially to form a 'hairpin' loop under and partly through the dorsal motor nucleus of the vagus in order to leave the medulla laterally. Particularly at rostral levels, however, this 'hairpin' extended medially almost to the midline (e.g. Figs. 1D, E, 2F–H).

(*c*) *Nucleus retroambiguus* (*nRA*). At the most caudal medullary and upper cervical spinal levels, the nucleus ambiguus was replaced by groups of neurons, discontinuous

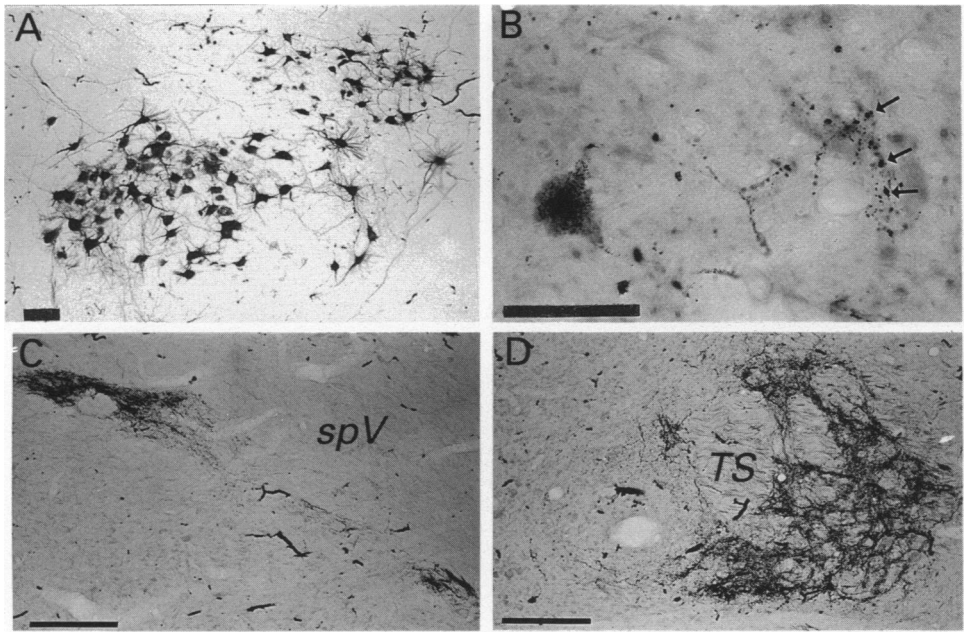

Fig. 8 (A–D). Bright-field photomicrographs showing in (A) retrogradely labelled nucleus ambiguus neurons; in (B), a single retrogradely labelled nucleus ambiguus neuron and presumed vagal afferent terminals (arrows); in (C) and (D) terminal labelling within the interpolated subnucleus of *spV* and the interstitial subnucleus of *nTS* respectively, following an injection of CTB-HRP into the superior laryngeal nerve. All depict the right side in transverse plane. Bars, (A) and (B), 0·1 mm; (C) and (D), 0·25 mm.

in the rostrocaudal direction, which occupied a position in the lateral part of the intermediate grey, immediately ventromedial to the lateral cervical nucleus (Figs. 1 M–P, 2 I–K, 9 A, B). At caudal medullary and upper C1 levels these neurons retained the multipolar morphology of more rostral ambiguus neurons, and in some sections were continuous medially with the most caudal DMN X neurons (e.g. Fig. 1 M). At lower levels, however, they became confined to the lateral intermediate grey and took on a bipolar appearance, with lateral processes which extended into the lateral funiculus, and medial processes which crossed the midline in discrete fascicles to terminate in the homologous nucleus of the contralateral side (Figs. 1 N, 2 I–K, 9). The axons of these peculiar neurons, like those of the more rostral ambiguus neurons, left the nucleus dorsally and made a hairpin loop before leaving the cord but, because of the reduced size of the 'reticular formation' at the base of the dorsal horn, the ascending component of the axonal trajectory was very short. The axons finally left the cord through the lateral funiculus under the head of the dorsal horn.

(*d*) *Nucleus dorsomedialis* (*ndm*). In all cases of ganglion injection, and in both control cases, a group of large, multipolar neurons was labelled at the dorsomedial margin of the ventral horn (Figs. 1 L–O, 9 B). These neurons had huge dendrites which ramified in several directions: dorsolaterally towards the nucleus retroambiguus; laterally and ventrolaterally into the lateral and ventral funiculi; dorsomedially into the ventral white commissure to cross the midline; medially to cross the midline through the medial part of the ventral funiculus. The last two sets of dendrites appeared to terminate in close proximity to ndm of the contralateral side. The axons of all these neurons left the cord via the ipsilateral ventral roots.

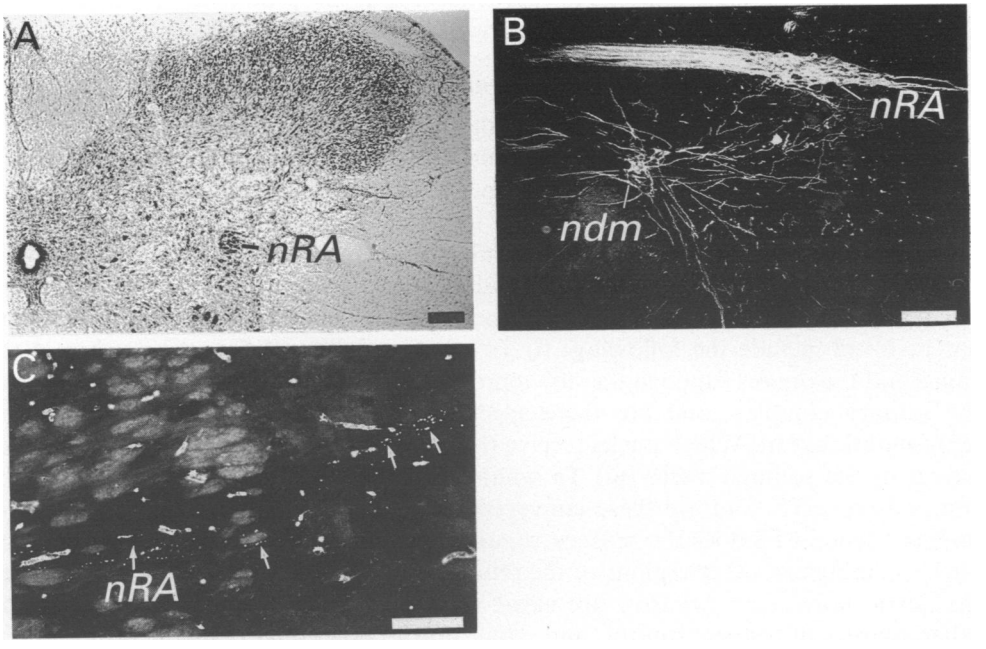

Fig. 9 (A–C). (A) Bright-field photomicrograph showing the nucleus retroambiguus (*nRA*) in the lateral intermediate grey of a C1 section (Nissl stain). (B) Dark-field photomicrograph showing retrogradely labelled *nRA* neurons and their processes extending laterally and medially. The medially directed ones are shown in the contralateral intermediate grey and *nRA* in (C) (arrows). Also shown in (B) are a few retrogradely labelled *ndm* neurons and their extensively ramifying dendrites. Bars, 0·25 mm.

An occasional cell was labelled in other, more ventrolateral, parts of the ventral horn (e.g. Fig. 1P).

DISCUSSION

Technical considerations

The technique of transganglionic labelling of vagal afferents, and the retrograde labelling of vagal efferents, by the injection of the cervical vagus with HRP or one of its conjugates, or exposure of its central cut end to such tracers, has been successfully employed by a host of workers in a variety of species (e.g. Kalia & Mesulam, 1980*a*; Wild, 1981; Katz & Karten, 1983; Nomura & Mizuno, 1983; Scharoun, Barone, Wayner & Jones, 1984; Gwyn *et al.* 1985; Miceli & Malmsbury, 1985; Hamilton *et al.* 1987; Altschuler *et al.* 1989). In the lamb, this technique has been much less successful, for reasons which are not clear. Injections into the nodose ganglion, however, consistently produce heavy, and even dramatic, central labelling both anterogradely and retrogradely, as was shown initially by Kalia & Mesulam (1980*a*) in the cat. This contrasts with findings in the dog, where injections of WGA-HRP into the nodose ganglion have been reported to label very few vagal efferent neurons (Chernicky *et al.* 1984). In the present study the difference in the degree of retrograde labelling between cervical vagus and nodose ganglion applications is particularly difficult to understand, because the efferent fibres would presumably be exposed to the tracer in a similar manner whether they were passing through the ganglion or through the cervical vagus. There is similarly no ready explanation for the fact that injections

into the superior laryngeal nerve were successful in producing transganglionic labelling, whereas those into the cervical vagus were generally not.

Vagal afferent projections

Norman & Bower (1982) noted that there had been nearly forty studies of the central projections of vagal visceral afferent fibres, and many of the examples cited in the present study have been published since then. This proliferation of studies of a single cranial nerve naturally reflects its importance in the sensory control of the viscera, but it also reflects in part the difficulty that investigators have had in resolving a complicated pattern of input to an equally complicated secondary sensory nucleus (i.e. nTS) in a wide variety of species. Some of the questions around which discussion has revolved include the following: (i) To what extent are different branches of the vagus and the organs supplied thereby represented differentially in individual nuclei of the solitary complex, and are there species differences in the specificity of these representations? (ii) Which nuclei receive the heaviest projections, e.g. those medial or lateral to the solitary tract? (iii) To what extent are there vagal projections to the contralateral nTS, and are these conveyed by contralateral TS fibres? (iv) To what targets outside nTS does the sensory vagus project, e.g. the area postrema, DMN X, nucleus ambiguus, other regions of the reticular formation, trigeminal sensory nuclei, the dorsal horn, etc.? (v) How are vagal afferent terminations related to vagal and other sources of visceral output, and what are the functional consequences of these patterns of connectivity for the sensorimotor control of the viscera? The present results are discussed in the context of these questions, and although they can speak to them only in a limited way, they nevertheless help to complete or consolidate our picture of the organisation of the vagal projections.

The question of sensory viscerotopy within nTS has been a particularly difficult one to resolve, perhaps because it can be viewed from either the perspective of the particular nTS subnucleus in question, or that of the organ innervated. That is to say, it can meaningfully be asked whether a particular nTS subnucleus contains the representation of a single organ (as against that of several organs) *and* whether a particular organ has its sensory representation confined to a particular nTS subnucleus (as against its representation being distributed throughout several subnuclei). In other words, the problem can be stated in terms of the degree of convergence and divergence of vagal projections upon the nTS subnuclei. Since there are more organs and tissues supplying afferents than there are recognised subnuclei, convergence of afferent projections onto some individual subnuclei is both a logical necessity and a demonstrated fact (e.g. Kalia & Mesulam, 1980*b*; Altschuler *et al.* 1989). Functionally, this convergence can in some instances be explained by the distribution of the same type of sensory receptor throughout several different tissues. Alternatively, it can be explained in other instances by the projection of different nerves innervating various types of receptors onto a single subnucleus, such as occurs via the glossopharyngeal and superior laryngeal nerves, innervating chemosensory and mechanosensory receptors in the caudal oral cavity, and projecting onto the interstitial subnucleus (Sweazy & Bradley, 1986; Altschuler *et al.* 1989; present results).

There is equally good evidence for the divergence of sensory projections from individual organs or vagal nerve branches to several nTS subnuclei (e.g. Kalia & Mesulam, 1980*b*; Sweazey & Bradley, 1986; Altschuler *et al.* 1989). Vagal afferents innervating certain visceral organs or tissues, however, appear to have a strong preference for terminating within a particular nTS subnucleus, e.g. the oesophagus within the central subnucleus (Katz & Karten, 1983; Fryscak, Zenker & Kantner,

1984; Altschuler *et al.* 1989), cardiovascular afferents within the dorsolateral subnucleus (Katz & Karten, 1979; Kalia & Mesulam, 1980*b*; Kalia & Welles, 1980), and gastric afferents within the subnucleus gelatinosus (Kalia & Mesulam, 1980*b*; Gwyn *et al.* 1985; Altschuler *et al.* 1989).

The present results are at variance with many previous studies of the projections of the cervical vagus or nodose ganglion, in that they show the lateral subnuclei (dnTS, dlnTS, vlnTS) to be consistently more heavily labelled than those subnuclei medial to the solitary tract such as the mnTS and subnucleus gelatinosus, even though they, too, were densely labelled. The significance of this finding is obscure, but nonetheless could indicate a very substantial projection from cardiovascular and respiratory structures, which have been found to project upon the lateral subnuclei in other animals (Kalia & Welles, 1980; Kalia & Mesulam, 1980*b*; Katz & Karten, 1979, 1983). In contrast, labelling of the lateral subnuclei in monkeys is conspicuous by its relative absence (Gwyn *et al.* 1985; Hamilton *et al.* 1987).

There seems to be general agreement that when projections are observed to the contralateral nTS they are concentrated medial to the solitary tract and within the commissural subnucleus and, to a lesser extent, in the dorsolateral subnucleus. This may reflect the preponderance of projections to these regions from midline or unpaired organs such as the heart to dlnTS, and the stomach to medial subnuclei, as Kalia & Mesulam (1980*b*) have suggested. The representation of another midline, unpaired structure, namely the oesophagus, is also bilateral with respect to each left and right vagus, but only to a slight extent. In the present study the central subnucleus of nTS, wherein sensory fibres innervating the oesophagus have been found to terminate in other animals (Katz & Karten, 1983; Fryscak *et al.* 1984; Altschuler *et al.* 1989), was very sparsely labelled contralateral to the side injected.

The source of projections to the contralateral nTS has not always been clear, partly because the contralateral TS has not always been observed to be labelled following injection of the ipsilateral cervical vagus or nodose ganglion (e.g. Kalia & Mesulam, 1980*a*). In the present study, the contralateral TS was distinctly labelled and undoubtedly gave rise to some of the terminal labelling within the contralateral nTS. However, it is possible that there are other sources of contralaterally directed fibres; the fibres heading medially under the hypoglossal nucleus, mostly rostral to the obex, could be one such source (Kalia & Mesulam, 1980*a*; present study: Results, Section 2*a*), although they were never actually observed to reach the contralateral side. Norman & Bower (1982) observed commissural fibres under the floor of the fourth ventricle rostral to the obex in the hen and these were suspected of giving rise to terminations in the contralateral medial reticular formation. A similar area of terminations was not observed in the present study.

Although nTS is the primary target of vagal afferents, there is ample evidence, including some from the present study, for vagal terminations in other brainstem nuclei. Two originally controversial, but now well documented, targets are the area postrema (AP) and the dorsal motor nucleus of the vagus (DMN X). In the present study the ipsilateral AP was densely labelled throughout its rostrocaudal and mediolateral extents, and the contralateral AP less so. In other species the AP has been reported to receive afferents from a variety of respiratory, cardiovascular and gastrointestinal sources (e.g. Kalia & Mesulam, 1980*b*; Kalia & Welles, 1980; Davies & Kalia, 1981; Odekunle & Bower, 1985; Fitzakerley & Lucier, 1988; Norgren & Smith, 1988). There is also some evidence for at least a crude form of viscerotopic representation within the AP (Norgren & Smith, 1988).

Vagal afferent terminations within DMN X have been reported by several workers

(e.g. Kalia & Mesulam, 1980*a*; Kalia & Sullivan, 1982; Chernicky *et al.* 1984; Miceli & Malmsbury, 1985; Odekunle & Bower, 1985; Fitzakerley & Lucier, 1988; present study), but not by others (e.g. Ciriello *et al.* 1981; Leslie *et al.* 1982; Norman & Bower, 1982; Hamilton *et al.* 1987). However, as Hamilton *et al.* (1987) have pointed out, this once controversial issue is now somewhat academic in view of the fact that the dendrites of DMN X neurons have been shown to extend into nTS (Shapiro & Miselis, 1985; Rinaman & Miselis, 1987), thereby providing the opportunity for monosynaptic vago-vagal reflexes.

Another controversial region of primary afferent terminations is the ventrolateral medulla, and/or the nucleus ambiguus. Davies & Kalia (1981), using the transganglionic HRP technique in the cat, showed that some carotid sinus nerve afferents terminated in the region of the nucleus ambiguus at levels straddling the obex, but it is believed that the present study is the first to report the termination of vagal afferents there. The interpretation is not entirely unequivocal because of the presence of retrogradely labelled ambiguus neurons in close proximity, which had extensively labelled dendrites; however the beaded nature of the presumed afferents and their association with labelled afferent fibres descending from TS, which were separate from exiting ambiguus axons, strongly supports their identity as vagal afferent terminations. The peripheral source of these afferents is not known, but it is interesting that evoked potentials have been recorded in a very similar region to that reported in the present study (i.e. dorsal and dorsomedial to the nucleus ambiguus at levels just caudal to the obex), subsequent to stimulation of the aortic nerve in the rabbit (Kumada & Nakajima, 1972), although these authors felt that the latency of the potentials (16–29 ms) was indicative of at least a disynaptic path via the nTS. It could be, however, that there is a region in the ventrolateral medulla close to the level of the obex which receives both carotid sinus and aortic nerve primary afferents.

Vagal afferents to parts of the nucleus of the spinal trigeminal tract (nspV) and the dorsal horn of upper cervical segments have been reported in some species (monkey: Rhoton, O'Leary & Ferguson, 1966; Beckstead & Norgren, 1979; Gwyn *et al.* 1985; Hamilton *et al.* 1987; cat: Kerr, 1962; Kalia & Mesulam, 1980*b*), but not in others (rat: Kalia & Sullivan, 1982; Leslie *et al.* 1982; dog: Chernicky *et al.* 1984; ferret: Odekunle & Bower, 1985). Those such as were observed in nspV in the present study formed dense patches, primarily in the nucleus interpolaris, corresponding closely to the position of vagal afferent terminations in the squirrel monkey (Gwyn *et al.* 1985). The origin of many of these afferents seems likely to be the larynx since they can be produced by exposure of the superior laryngeal nerve to HRP (cat: Nomura & Mizuno, 1983; lamb: Sweazey & Bradley, 1986; present study), although they have also been reported following injections into the cervical vagus (monkey: Gwyn *et al.* 1985). In the present study, terminations in the dorsal horn were found to be limited to Lamina I; in monkeys terminations are found in deeper laminae (Rhoton *et al.* 1966; Gwyn *et al.* 1985).

Very distinct patches of terminations were also observed in the principal trigeminal sensory nucleus (PrV) in the present study after both nodose ganglion and superior laryngeal nerve injections, confirming previous anatomical and electrophysiological reports of superior laryngeal nerve projections in the lamb, sheep and cat (Sweazey & Bradley, 1986; Car, Jean & Roman, 1975; Jean, Car & Roman, 1975; Nomura & Mizuno, 1983). These projections in the lamb and sheep could be involved in a gustatory pathway to the thalamus (Car *et al.* 1975; see Sweazey & Bradley, 1986).

The last set of afferent terminations to be discussed is also the most novel. In the lamb a distinct terminal field, supplied by labelled fibres which could be traced

rostrally and dorsolaterally from the level of PrV, was observed lateral and ventral to the caudal superior cerebellar peduncle (scp), in regions we suggest are the lateral parabrachial and Kölliker–Fuse nuclei. No labelled fibres or terminations were observed medial to scp. Kalia & Kane-Wanger (1985) reported a similar vagal projection in the monkey (but also including the medial parabrachial nucleus), but this has not been fully documented and was not confirmed by Hamilton *et al.* (1987) in the same species (squirrel monkey). The peripheral source of this projection in the lamb is unknown. Superior or recurrent laryngeal nerve projections do not appear to reach these rostral levels (present study; Sweazey & Bradley, 1986; Nomura & Mizuno, 1983), so that they are likely to arise in more caudal regions. It may be of related interest that, in the fetal lamb, pontine lesions which include the parabrachial and Kölliker–Fuse nuclei abolish the hypoxic depression of breathing (Gluckman & Johnston, 1987). Whether this implies a respiratory component of the primary vagal input to these nuclei remains to be determined. The lateral parabrachial and Kölliker–Fuse nuclei in the rat have recently been shown to receive distinct projections from nTS subnuclei associated with respiratory input (Herbert, Moga & Saper, 1990).

Vagal efferent projections

A detailed analysis of the cytoarchitectural and viscerotopic organisation of vagal efferent neurons in the lamb and sheep must await future studies comparable with those in other species, which have begun to unravel the complexities of visceral preganglionic and somatic representation by treatment of individual nerves and organs (e.g. Fox & Powley, 1985; Katz & Karten, 1985; Bieger & Hopkins, 1987). As in other species, vagal efferent neurons in the lamb were predominantly located in one of two major rhombencephalic cell groups, the dorsal motor nucleus of the vagus (DMN X) or the nucleus ambiguus (nA). A few vagal neurons were scattered between the two, along the trajectory of ambiguus efferent fibres, and specific clusters of neurons extended caudally into the upper cervical spinal cord where they formed either a caudal continuation of DMN X medially, or the nucleus retroambiguus (nRA) laterally. Periodically, medial and lateral clusters were in continuity.

A striking feature of the nA in the lamb was the apparently large size of its rostral portion where the neurons were grouped into a more medial, densely clustered aggregate and a more lateral, looser collection. These may correspond to the compact and external divisions of the rostral nucleus ambiguus in the rat which supply special and general visceral efferent fibres innervating the oesophagus and supradiaphragmatic structures respectively (Bieger & Hopkins, 1987). However, the oesophageal specialisations of the ruminant may be reflected in a more complex organisation of oesophagomotor ambiguus neurons than is present in the rat. In this context it will be of interest to determine the pattern of connections between the central subnucleus of nTS, which probably receives oesophageal afferents as in other species (Katz & Karten, 1983; Fryscak *et al.* 1984; Altschuler *et al.* 1989) and the oesophageal motoneurons, upon which the central subnucleus specifically projects (Cunningham & Sawchenko, 1989).

The nucleus retroambiguus (or retroambigualis) occupies a similar position at lower medullary and upper cervical spinal levels, to that which the intermediolateral nucleus occupies at thoracic levels (Olszewski & Baxter, 1954; Taber, 1961). Like the latter, the caudal (upper cervical) portion of nRA in the lamb has neurons extending towards the midline at intermittent rostrocaudal levels, and these are sometimes in mediolateral continuity with the most caudal DMN X neurons. The most striking thing about the caudal nRA neurons in the lamb, however, is the orientation, extent

and fasciculation of their presumed dendrites, one group extending laterally into the lateral funiculus and another extending medially and contralaterally to reach the nRA of the opposite side. Since the axons of these neurons leave the cord laterally, under the head of the dorsal horn, there is a possibility that the dendrites of nRA neurons of one side make contact with nRA cell bodies of the opposite side. The pre- or postsynaptic nature of any such contacts would be interesting to determine. The functional nature of the crossed dendritic projections may be related to the possibility that caudal nRA neurons innervate midline, unpaired organs such as the larynx, trachea and stomach, as they do in the cat (Kalia & Mesulam, 1980*b*).

Other labelled neurons in the lamb upper spinal cord with extensive and crossed dendrites lay in the nucleus dorsomedialis (ndm), but these were also labelled in both control cases and their axons were clearly seen to leave the cord through the ventral root rather than through the lateral funiculus. These facts strongly suggest that ndm neurons do not project their axons into the vagus in the lamb, and that they were inadvertently labelled by leakage of tracer to adjacent neck muscles. This is supported by investigations of neck muscle innervation in several species (see Callister, Brichta & Peterson, 1987 for review). Neurons of ndm have been labelled following HRP treatment of the cervical vagus or nodose ganglion in the cat (Kalia & Mesulam, 1980*a*) and monkey (Gwyn *et al.* 1985; Hamilton *et al.* 1987), but not in the rat (Kalia & Sullivan, 1982). In the monkey they were labelled bilaterally (Gwyn *et al.* 1985) and in the cat the only peripheral organ injection giving rise to ndm labelling was into the extrathoracic trachea (Kalia & Mesulam, 1980*b*). Again, these findings could suggest that ndm innervates neck muscles rather than supplying the vagus, although ndm neurons were not labelled in Kalia & Mesulam's (1980*a*) control cases.

A small number of neurons in the spinal accessory nucleus have also been reported to project their axons into the cervical vagus in the cat (Kalia & Mesulam, 1980*a*), rat (Kalia & Sullivan, 1982) and monkey (Gwyn *et al.* 1985), but no labelled neurons in either the medial or lateral divisions of this nucleus (Callister *et al.* 1987; Krammer *et al.* 1987) were unequivocally identified in the present study.

SUMMARY

Injections of WGA-HRP and CTB-HRP were made into the cervical vagus or the nodose ganglion in a series of lambs, in order to define the sensory projections and motor origins of the vagus nerve. Injections into the nodose ganglion were much more successful than injections into the cervical vagus in effecting the desired result. The former produced labelling of both descending and ascending components of the solitary tract (TS). The descending component terminated massively in all ipsilateral and certain contralateral subnuclei of the nucleus of the solitary tract (nTS) and in the upper cervical spinal cord. Patchy terminations were also observed within the interpolated subnucleus of the nucleus of the spinal trigeminal tract, and within Lamina I of the upper cervical cord. The ascending component of TS terminated in rostral regions of the nTS, and in specific portions of the principal sensory trigeminal nucleus and the lateral parabrachial and Kölliker–Fuse nuclei.

The motor origins of the vagus nerve arose almost completely ipsilaterally in the dorsal motor nucleus of the vagus, the nucleus ambiguus, and the caudal portion of the nucleus retroambiguus situated in the lateral part of the intermediate grey at upper cervical spinal levels. Labelled neurons in the nucleus dorsomedialis of the upper spinal cord were thought not to project their axons into the cervical vagus.

The work was supported by grants from the Medical Research Council of New Zealand to all three authors. The histological assistance of Mrs S. M. Braan-Stroo is gratefully appreciated.

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ABBREVIATIONS

AP, Area postrema; *C*, cuneate nucleus; *cc*, central canal; *dcn*, dorsal cochlear nucleus; *G*, gracile nucleus; *icp*, inferior cerebellar peduncle; *K-F*, Kölliker–Fuse nucleus; *LC*, locus coeruleus; *mcp*, middle cerebellar peduncle; *mlf*, medial longitudinal fasciculus; *mV*, motor nucleus of the trigeminal nerve; *nA*, nucleus ambiguus; *nCL*, lateral cervical nucleus; *ndm*, nucleus dorsomedialis; *nRA*, nucleus retroambiguus; *nTS*, nucleus of the solitary tract; *cnTS*, central subnucleus; *dnTS*, dorsal subnucleus; *dlnTS*, dorsolateral subnucleus; *mnTS*, medial subnucleus; *ncom*, commissural subnucleus; *sg*, subnucleus gelatinosus; *vnTS*, ventral subnucleus; *vln TS*, ventrolateral subnucleus. *NV*, trigeminal nerve; *nVI*, abducens nucleus; *NVII*, facial nerve; *nX*, dorsal motor nucleus of the vagus nerve (DMN X); *nXII*, hypoglossal nucleus; *nPBl*, lateral parabrachial nucleus; *PH*, perihypoglossal nucleus; *PrV*, principal sensory nucleus of the trigeminal nerve; *RtL*, lateral reticular nucleus; *spV*, spinal tract of the trigeminal nerve; *scp*, superior cerebellar peduncle; *TS*, solitary tract: *ATS*, ascending solitary tract; *DTS*, descending solitary tract. *VeD*, descending vestibular nucleus; *VeL*, lateral vestibular nucleus; *VeM*, medial vestibular nucleus.