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Fibre composition of the hypoglossal nerve in the rat*

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INTRODUCTION

In the rat, the hypoglossal (XII) nerve is motor to the extrinsic and intrinsic muscles of the tongue and to the geniohyoid muscle. The nucleus of origin of this nerve extends the full length of the medulla oblongata and comprises dorsal and ventral nuclear tiers. The dorsal tier contributes axons to the lateral division of the peripheral XII trunk, the ventral tier to the medial division (Krammer, Rath & Lischka, 1979). The extrinsic muscles are supplied by both divisions, the intrinsic muscles and geniohyoid by the medial division only (Uemura-Sumi, Itoh & Mizuno, 1988).

The ventral nuclear tier divides at its caudal end into medial and lateral subnuclei. Kitamura and his co-workers claim that axons from each subnucleus reach the peripheral XII trunk indirectly, by way of the first cervical ventral root and the ansa cervicalis. They claim, further, that those from the medial subnucleus are destined for the supply of some intrinsic muscle fibres in the tongue, and that those from the lateral subnucleus contribute an accessory supply to the geniohyoid (Kitamura, Nichiguchi & Satai, 1983, 1985; Kitamura *et al.* 1986). Their evidence is based on results of retrograde labelling of the XII nucleus following application of horseradish peroxidase (HRP) to branches of the XII nerve or to the ansa cervicalis; they did not examine the muscles. The validity of their claims has been assessed in the present study by combining the results of retrograde labelling with those of anterograde degeneration within the relevant muscles.

The geniohyoid muscle is of particular interest because it consistently possesses neuromuscular spindles (Maier, 1979). Since proprioceptive fibres contained in the distal XII trunk of the rat are derived (at least mainly) from cervical dorsal root ganglion cells (Neuhuber & Mysicka, 1980), the question arises whether the fusimotor and the afferent innervation of geniohyoid spindles travel by separate pathways.

Finally, Smith (1989) claims to have identified muscle spindles in the inferior longitudinal muscle of the rat tongue. The numbers were small (0, 2, 4 and 7 spindles, respectively, in each of four tongues), and her finding runs counter to the prevailing belief that spindles are absent from the tongue in mammals below the primate level. Smith's claim has been briefly explored in the present work.

MATERIALS AND METHODS

Sixty three Sprague–Dawley rats (150–300 g) were used. For all the experimental and fixation procedures, anaesthesia was introduced by intra-abdominal injection of chloral hydrate (0·75 g/kg). Relevant nerves were approached through an incision made either in the midline or close to the mandibular margin. Nerve sections were performed with the aid of a Zeiss operating microscope. Following a postoperative

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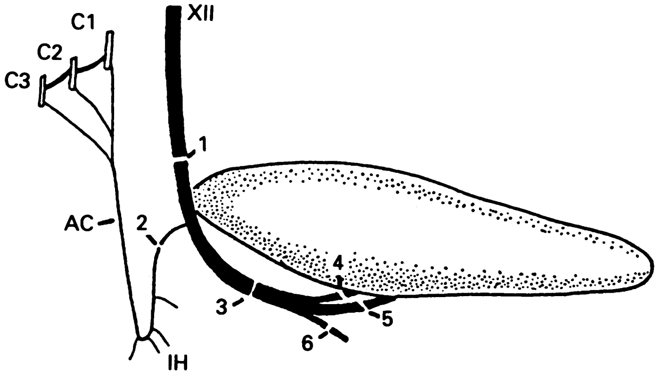


Fig. 1. Plan of the rat hypoglossal nerve (XII) and ansa cervicalis (*AC*). Numbers indicate sites of fluorescent dye application to proximal faces of cut nerves: 1, proximal XII trunk; 2, ansa cervicalis distal to its four infrahyoid (*IH*) branches; 3, distal XII trunk; 4, lateral, 5, medial division of XII nerve; 6, geniohyoid branch.

survival period, an overdose of chloral hydrate was administered prior to systemic perfusion with fixative.

Retrograde labelling

Surgical procedures

(*a*) In 15 rats, crystals of FluoroGold (FG, Fluorochrome Inc, Englewood, Colorado) or Fast Blue (FB, Illing GmbH, F.R.G.) were applied to a transected nerve at one or either of the sites shown in Figure 1. Each time, the proximal stump was first mounted on a piece of plastic film; the crystals were crushed into the nerve tip with a pair of fine forceps and the preparation was left undisturbed for one hour; the entire site was irrigated with saline and dried with cotton swabs; the nerve tip was sealed with cyanoacrylate and the plastic film was removed before wound closure. In some experiments either the proximal XII trunk (above the ansa cervicalis connection) or the ansa was cut immediately before the dye was applied to the distal XII trunk or to its branch to geniohyoid. In three instances, the trunk of the facial nerve was severed close to its foramen of exit from the skull; in another two, the lingual nerve was severed at its point of entry into the tongue; in all five, dye was then applied to the distal cut XII trunk.

(*b*) In 15 rats, 1 mg FG or FB was made into a paste with water and used to coat a root canal reamer (Kleuber & Ontell, 1984) which was inserted at several sites along both sides of the tongue (about 5 mg dye *in toto*). In four of these rats the distal XII trunk was severed prior to insertion of dye into the tongue; in three, the lingual nerve was severed instead and in another three the facial nerve instead.

(*c*) Combination labelling experiments were also carried out as follows: (*i*) In five animals the facial nerve trunk was injected close to the stylomastoid foramen with Nuclear Yellow (NY, Illing GmbH, F.R.G.) solution (1 mg in 0·1 ml water) and the distal cut XII trunk was treated with FB in the usual way. (*ii*) In a further five, NY was applied to the proximal face of the lingual nerve, the nerve having been transected at its point of entry into the tongue. (For access to the lingual nerve, and to the branches of XII shown in Figure 1 for other experiments, the ipsilateral mylohyoid muscle was first cut and reflected.)

Fixation and staining procedures

Following a survival period of 2–4 days, the rats were perfused through the ascending aorta with 20 ml saline followed by 200 ml neutral 10% formal saline containing 15% sucrose. The calvaria was opened and the brainstem exposed before immersion of the head and neck in the same fixative overnight. Serial transverse sections of the pons, medulla oblongata, and upper spinal cord were taken on a freezing microtome at 30 μm. The sections were mounted on slides, air-dried and examined in a Leitz Laborlux K microscope with suitable absorption and excitation filters. Some were later stained with 0·05% methylene blue (Sripanidkulchai & Wyss, 1986) or 1% thionin. In relevant animals the three uppermost cervical dorsal root ganglia, the jugular-nodose ganglion complex of the vagus and the superior cervical ganglion were sectioned and processed in the same manner.

Anterograde degeneration

Surgical procedures

In order to detect any ansa cervicalis fibres within the tongue, proximal XII nerve section was combined with section of the lingual nerve on the same side at its point of entry into the tongue. This double nerve section was carried out in nine rats. In six others the proximal XII nerve alone was cut and in a further six, the distal XII trunk alone. In all cases each distal nerve stump was turned aside in order to retard regeneration.

Fixation and staining procedures

Following a survival interval of 2–14 days, all of these rats were perfused with 5% paraformaldehyde containing 3·4% sucrose and made neutral with sodium sulphite. After storage for two days at 4 °C, horizontal, coronal or sagittal sections of the tongue were taken at 40 μm and reacted for cholinesterase activity by the method of Karnovsky & Roots (1964). Horizontal or sagittal sections of the two geniohyoids were also reacted. A week later, all sections were impregnated with silver by a modification of FitzGerald's (1963) Gros–Bielschowsky technique.

Identification of muscle spindles

Two unoperated rats were perfused with Bodian's (1937) fixative; serial paraffin sections of the entire tongue and geniohyoid were taken at 15 μm in the coronal plane and impregnated with protargol. In order to examine the assertion by Smith (1989) that occasional spindles are to be found in the posterior part of the inferior longitudinal muscle of the tongue, the posterior one third of a further six control tongues (including the geniohyoid) was immersed in Bodian's fixative; serial paraffin sections were taken at 10 μm and stained by van Gieson's method.

RESULTS

Gross anatomy

The extracranial XII nerve runs in front of the delicate carotid sheath before passing ventrally, under cover of the digastric muscle, to reach the submandibular region. It splits into a larger medial and a smaller lateral division before passing above the mylohyoid. The medial division gives an immediate branch to the geniohyoid, then expends itself in numerous twigs to the genioglossus and to the intrinsic muscles of the tongue. The lateral division supplies the styloglossus and hyoglossus.

While in front of the carotid sheath, the XII nerve overlies the jugulo-nodose ganglion of the vagus nerve and it is linked to the ganglion by one or two very fine nerve strands. At this level it is also linked by nerve strands to the superior cervical

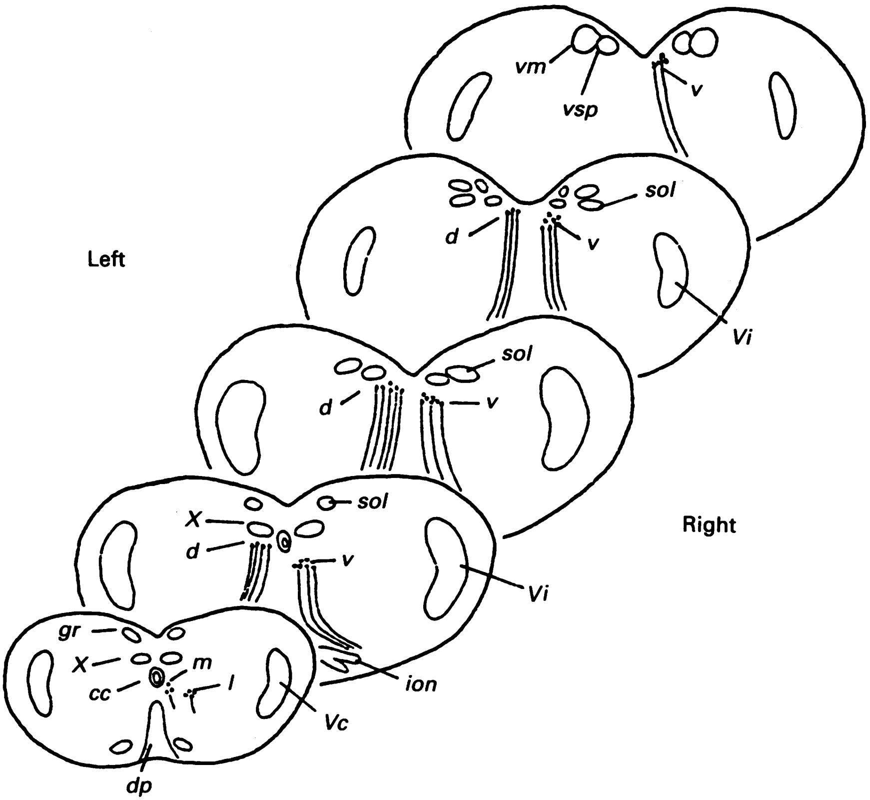


Fig. 2. Representative sections of medulla oblongata, showing XII nuclear and axonal labelling following application of Fast Blue to the lateral division of the left XII nerve and of FluoroGold to the medial division of the right XII nerve. XII nuclear elements: *d*, *v*, dorsal and ventral nuclear tiers; *l*, *m*, lateral and medial subnuclei of ventral nuclear tier. *Vc*, *Vi*, caudal and interpolar elements of spinal trigeminal nucleus; *X*, dorsal motor nucleus of vagus; *cc*, central canal; *dp*, decussation of pyramids; *ion*, inferior olivary nucleus; *sol*, nucleus solitarius; *vm*, *vsp*, medial and spinal nuclei of vestibular nerve.

sympathetic ganglion. Where it crosses the tip of the hyoid bone, the XII nerve receives a connection from the ansa cervicalis after the latter has distributed its branches to the infrahyoid muscles. The more distal branches of the medial division interdigitate with branches of the lingual nerve, which sweeps around the side of the tongue.

The ansa cervicalis of the rat is described in detail by Müntener *et al.* (1980). It arises by union of branches from spinal nerves C1, C2 and C3. C1 is a purely motor nerve, having no dorsal nerve root or ganglion (Neuhuber & Mysticka, 1980).

Retrograde labelling

The entire length of the ventral XII nuclear tier, including the medial and lateral subnuclei at the caudal end, was labelled by dye applied to the medial division of the peripheral XII nerve. The entire dorsal tier was labelled by dye applied to the lateral division (Fig. 2). Application of dye to the distal XII trunk, combined with section of the proximal XII trunk, resulted in labelling of the entire medial subnucleus of the ventral tier; a minority (two or three) of the cells were labelled in the lateral subnucleus. On the other hand, dye application to the XII branch to the geniohyoid resulted in complete labelling of the lateral subnucleus alone and, if the ansa cervicalis was cut at the same time, most (8–10), but not all, cells in the lateral subnucleus were

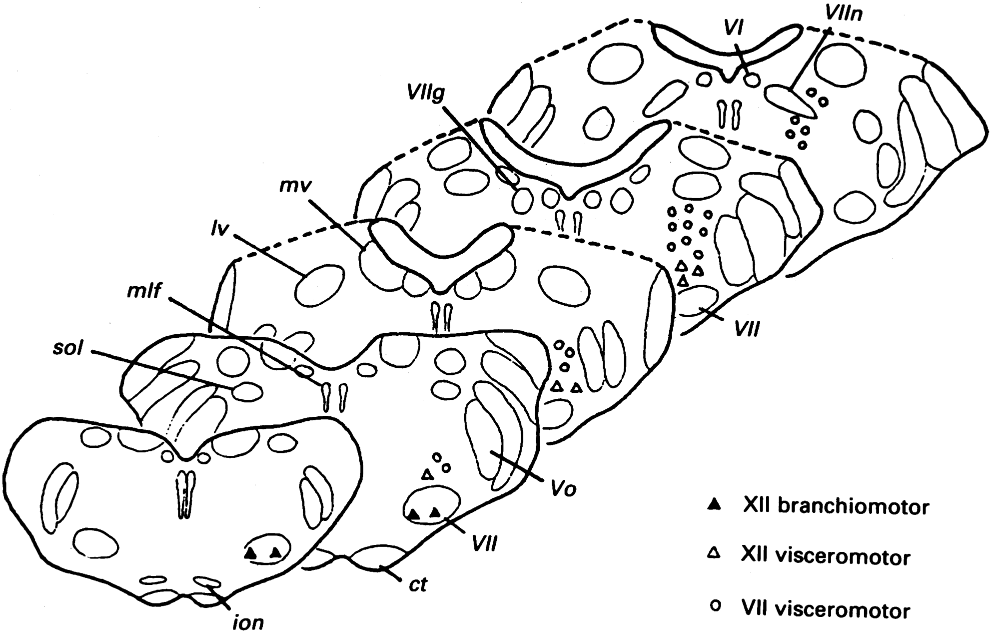


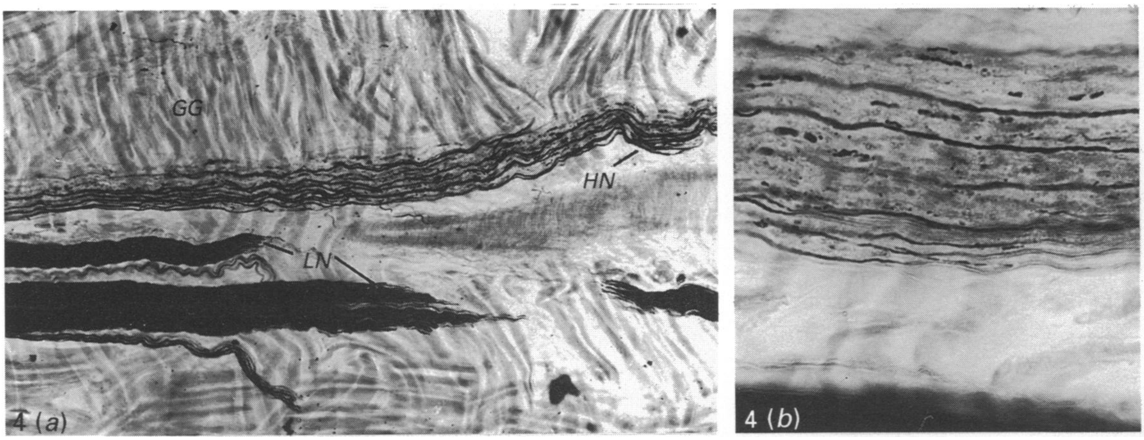
Fig. 3. Representative sections of pons showing locations (triangles) of cells contributing to the intramedullary XII nerve from the main facial nucleus and the salivatory nucleus. The circles indicate salivatory cells supplying intralingual ganglia via the chorda tympani. *Vo*, pars oralis of trigeminal spinal nucleus; *VI*, abducens nucleus; *VII*, facial nucleus; *VIIg*, genu of the *VIIn*, facial nerve; *ct*, corticospinal tract; *ion*, inferior olivary nucleus; *lv*, lateral vestibular nucleus; *mlf*, medial longitudinal fasciculus; *mv*, medial vestibular nucleus; *sol*, nucleus solitarius.

labelled. After dye application to the ansa cervicalis distal to the point of origin of its branch to the thyrohyoid muscle, all of the medial subnucleus was labelled, together with a few cells in the lateral subnucleus. Finally, application of Fast Blue to the distal XII trunk, together with FluoroGold application to the ansa cervicalis, resulted in an admixture of blue and gold fluorescence in the lateral subnucleus, with a predominance of blue cells; all of the medial subnuclear somata were gold.

The *main facial nucleus* contained 8–12 labelled cells, whether dye was applied to the cut proximal or distal XII trunk or inserted into the tongue (Fig. 3). This was a consistent finding even with prior transection of the ipsilateral facial or lingual nerve. However, it was not observed ipsilaterally if the XII nerve was cut prior to dye insertion into the tongue. No double-labelling was seen after application of FB to the hypoglossal nerve and NY to the facial nerve; instead, the FB-containing cells were interspersed among dozens of NY-filled cells in the lower part of the pons, occupying the ventromedial and ventrolateral subnuclei of the main facial nucleus, as delineated by Shohara & Sakai (1983) and Friauf (1986).

Unexpectedly, up to a dozen neurons were labelled in the *reticular formation of the pons*, following dye insertion into the tongue or application to the cut XII nerve (Fig. 3). Double labelling via the lingual nerve (NY) and the hypoglossal nerve (FB) enabled the XII-affiliated cells to be assigned to the salivatory nucleus. The lingual-connected cells were more numerous and lay dorsal to the XII-related cells. No double-labelling of individual somata was seen.

Following dye application intralingually, or to the distal XII trunk, or to the ansa cervicalis, up to 20 unipolar cells were labelled in C2 and C3 *dorsal root ganglia*. In the same experiments, 6–12 unipolar labelled cells were consistently seen in the *jugulo-nodose ganglion* complex, in the zone of mergence of the two component ganglia



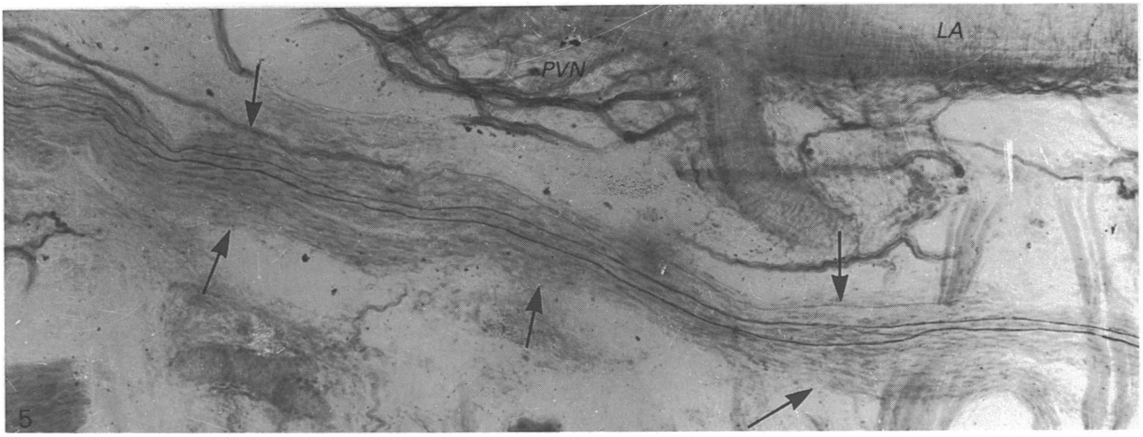


Fig. 4(*a–b*). (*a*) Sagittal section of tongue, fixed 4 days after proximal XII nerve section. *GG*, genioglossus; *HN*, branch of hypoglossal nerve; *LN*, branches of intact lingual nerve. Cholinesterase-silver. × 180. (*b*) Enlargement of a hypoglossal branch, showing persistent healthy axons scattered among degenerating XII nerve fibres. The persistent axons were nearly all myelinated. × 640.

Fig. 5. Sagittal section of tongue base, fixed 10 days after combined section of lingual and proximal XII nerves. The arrows indicate the margins of a large, degenerate branch of the medial division of XII, containing two healthy myelinated axons. *LA*, lingual artery; *PVN*, perivascular neuronal network of presumed autonomic origin. Cholinesterase-silver. × 150.

About a dozen labelled multipolar cells were scattered through the *superior cervical ganglion*, without any evidence of somatotopic distribution.

Anterograde degeneration

Tongue

As previously observed in several laboratories, the terminal distribution of the XII nerve was essentially ipsilateral. Following distal XII nerve section, minor transmedian overlap (0·5 mm or less) occurred only in the anterior and posterior ends of the tongue where transversus linguae muscle fibres interdigitated across the midline.

Following XII nerve section proximal to the connection with the ansa cervicalis, scattered myelinated fibres persisted in both divisions of the nerve but mainly in the medial division (Fig. 4). The surviving axons were seen to best advantage when the lingual nerve had been sectioned as well and the products of degeneration allowed to clear during a 5–7 day survival period (Fig. 5). In coronal sections, the axons were seen

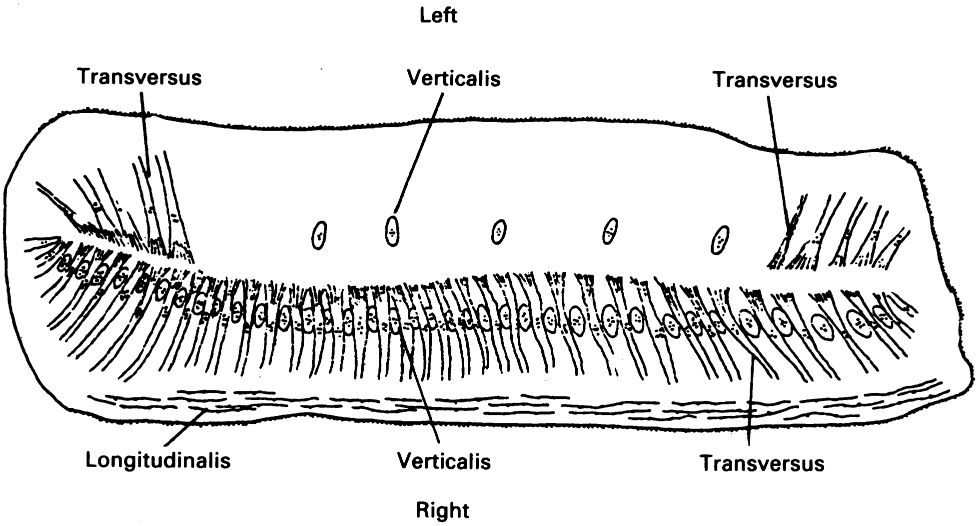


Fig. 6. Graphic reconstruction from camera lucida tracings of 4 serial frozen sections through the dorsal part of a tongue (anterior end to the right), fixed 5 days after section of the left proximal XII trunk. Only innervated muscle fascicles are shown. (The longitudinalis was completely denervated on the left side at this level.)

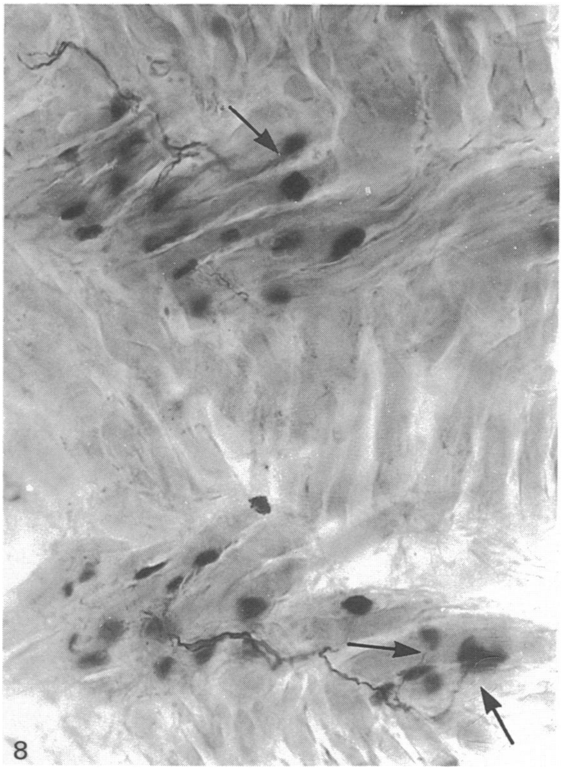
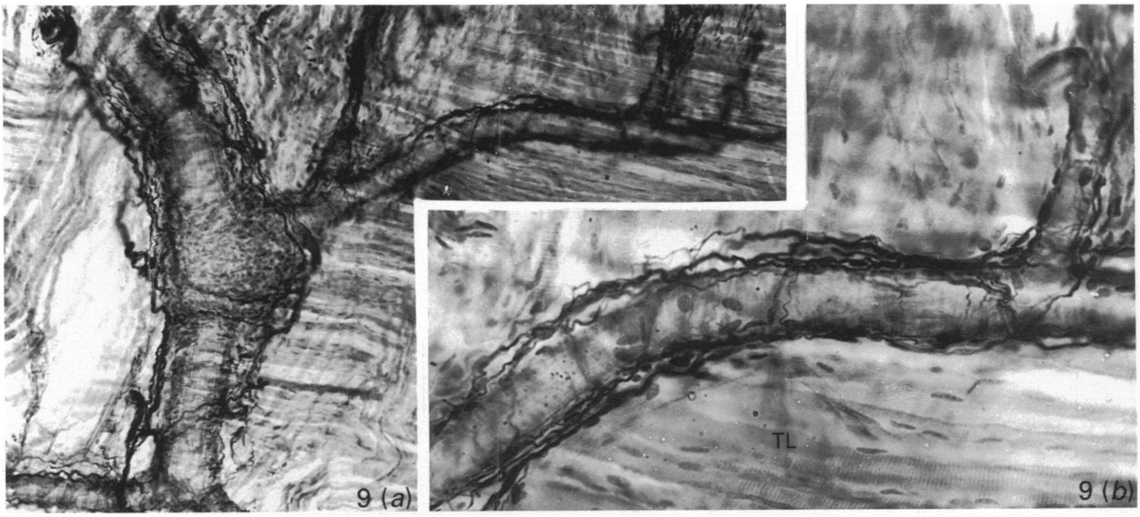


Fig. 7. Horizontal section through transversus linguae in the upper, anterior tongue, fixed 10 days after ipsilateral interruption of the proximal XII trunk. All of the muscle fibres had reactive motor end plates, but only about half of those in this field were innervated. Arrows indicate 2 innervated plates. Cholinesterase-silver. ×400.

Fig. 8. Field adjacent to Fig. 7, showing reactive end plates on verticalis muscle fibres. Only about one fifth of the plates in this field were innervated. Arrows indicate 3 innervated plates. ×400.



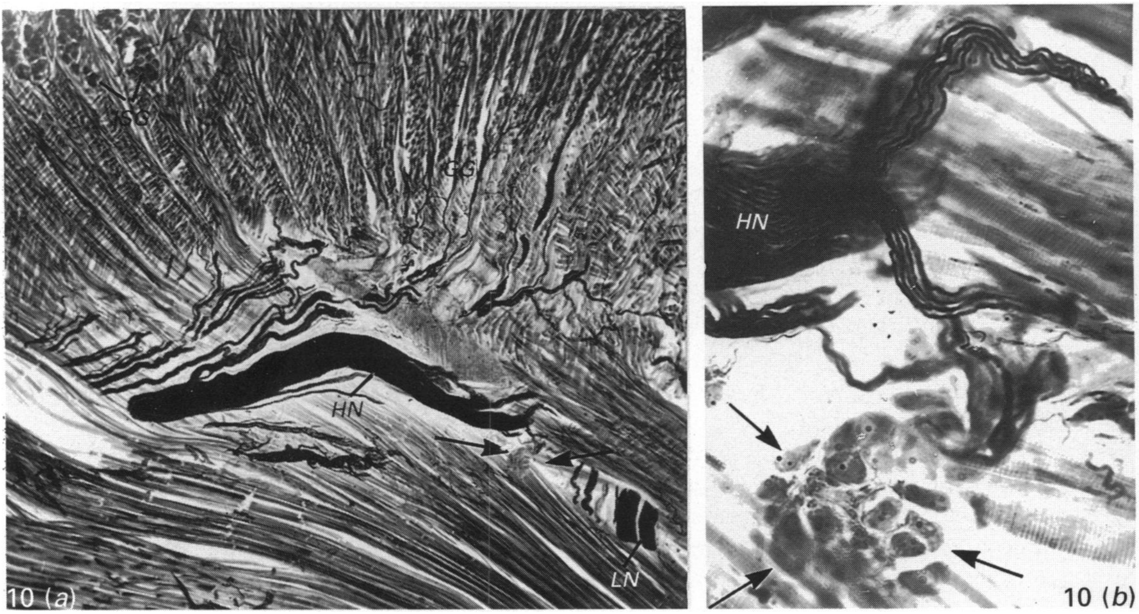


Fig. 9(*a–b*). (*a*) Coronal section, control side of posterior tongue, showing abundant, coarse and fine perivascular nerve fascicles accompanying branches of the lingual artery. Cholinesterase-silver. × 140. (*b*) Enlargement from Fig. 9(*a*). *TL*, transversus linguae. × 330.

Fig. 10(*a–b*). (*a*) Sagittal section, control side of posterior tongue, showing the position of a small ganglion (arrow) attached to a branch of the hypoglossal nerve (*HN*). *GG*, genioglossus; *ISG*, intralingual salivary glands; *LN*, lingual nerve branches entering the tongue. Cholinesterase-silver. × 40. (*b*) Enlargement from 10(*a*), showing ganglion cells (arrows). *HN*, branch of hypoglossal nerve. × 320.

to leave degenerated branches of the medial division by running upwards independently between fascicles of the verticalis linguae muscle. In horizontal sections, the great majority were seen to terminate in motor end plates on transversus linguae muscle fibres (Figs. 6, 7); the remainder terminated in end plates on verticalis (Figs. 6, 8) or longitudinalis superior. Persistent end plate innervation was seen in all of these experiments and was confined to the intrinsic musculature. It was more prevalent in the anterior part of the tongue than elsewhere. It was not seen in the posterior

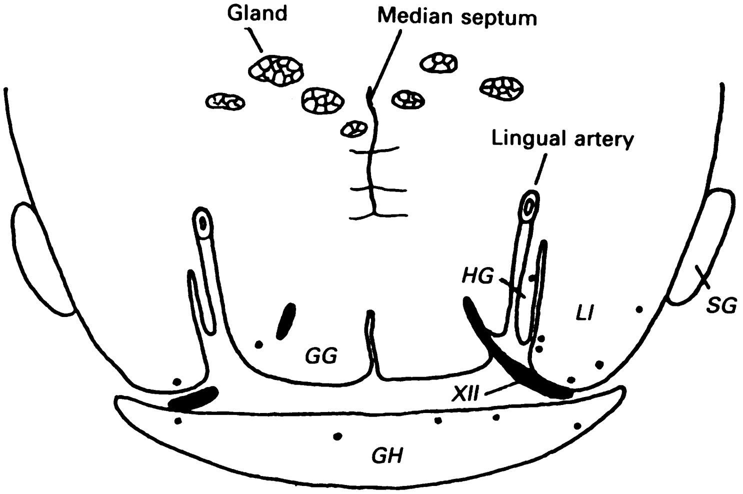


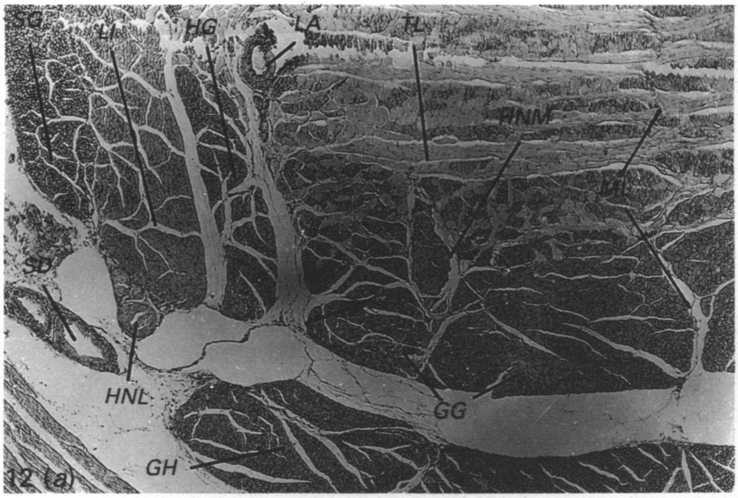
Fig. 11. Profile of a single, representative coronal section of the posterior tongue, giving the positions of 8 neuromuscular spindles in the tongue base. The material has been pooled from 5 animals. The geniohyoid muscles (*GH*) contained a total of 5 spindles in these sections. *GG*, genioglossus; *HG*, hyoglossus; *LI*, longitudinalis inferior; *SG*, styloglossus; *XII*, medial division of hypoglossal nerve.

extremity, where the bulk of the tongue is made up of intralingual salivary glands and extrinsic muscles.

The relative proportions of the total intrinsic musculature supplied by axons surviving proximal XII section varied between animals: for the transversus linguae, it varied from about one tenth to one quarter; for the other two muscles it never exceeded 5 per cent. In this context, sagittal sections of the tongue could be misleading because slight muscle wasting on the side of operation could be sufficient to impart a concavity to the median septum towards that side (as shown by coronal sections of similar specimens) with the result that fully innervated verticalis and longitudinalis superior muscle fascicles would be seen, apparently on the side of operation.

In addition to somatomotor fibres, both divisions of the degenerated XII nerve and some of their main branches contained myelinated fibres which lost their myelin sheaths upon bifurcating repeatedly in the connective tissue nearby. These branching fibres were more numerous on the contiguous surfaces of the hyoglossus and genioglossus than elsewhere. Some gained the adventitial coat of the lingual artery, or of its branches, there to mingle with abundant perivascular unmyelinated fibre bundles of presumed autonomic origin (Figs. 5, 9). A few terminated as fine branchlets in the median septum of the tongue. All of these fibres were regarded as afferent in nature. None could be traced to neuromuscular spindles or to classical tendon endings.

Numerous small ganglia were observed close to major nerve branches along the inferior aspect of the tongue. They were considered to be parasympathetic in nature because they were strongly cholinesterase-reactive and exhibited the same morphology as the ganglia adjacent to intralingual salivary glands which, in the rat, occupy the posterior one fourth of the tongue and belong to the glossopharyngeal nerve. In the anterior part of the tongue, postganglionic fibre bundles could be traced to branches of the lingual artery. The degeneration experiments showed clearly that the great majority received preganglionic fibres from the lingual nerve. However, a small number received them from the hypoglossal. On the control side, occasional ganglia were seen suspended from primary branches of the medial XII division, posterior to the site of entry of the lingual nerve (Fig. 10).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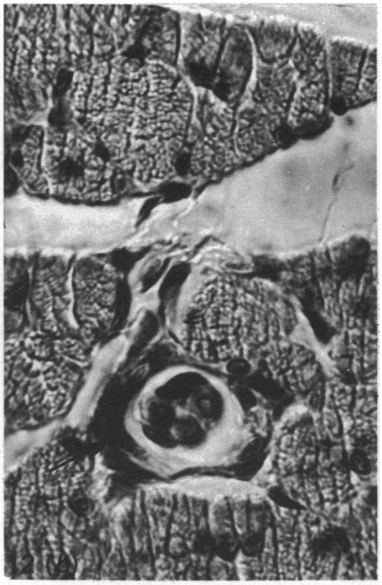
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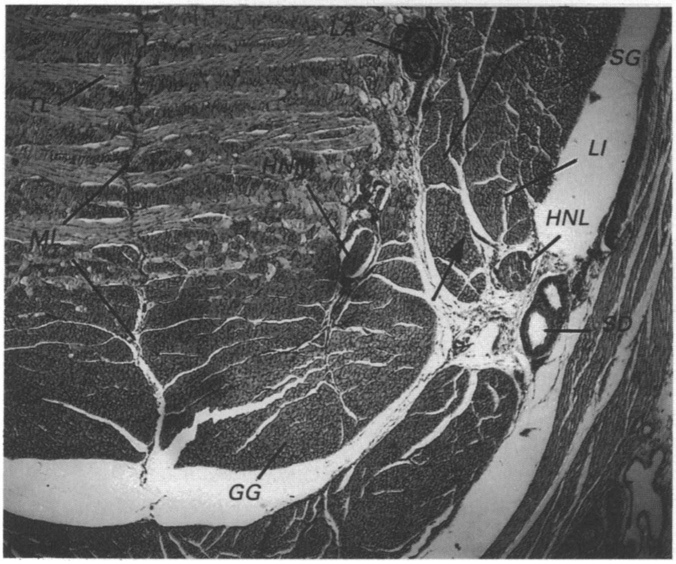
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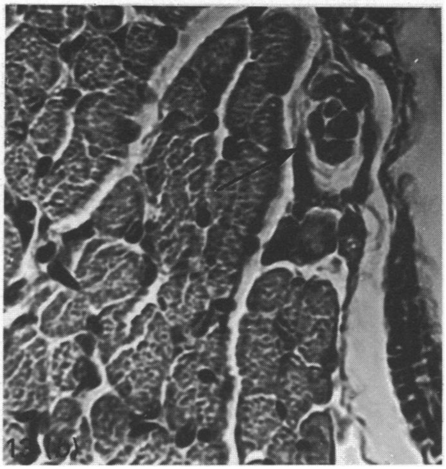
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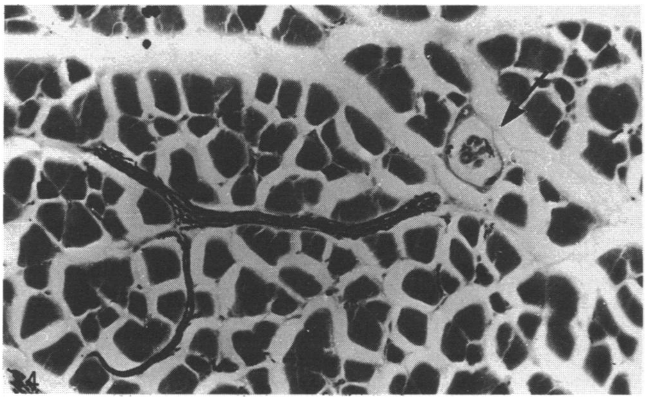
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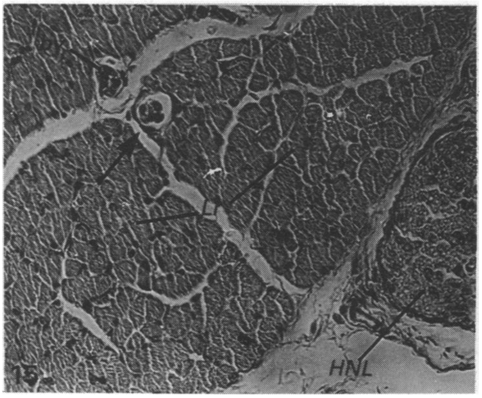
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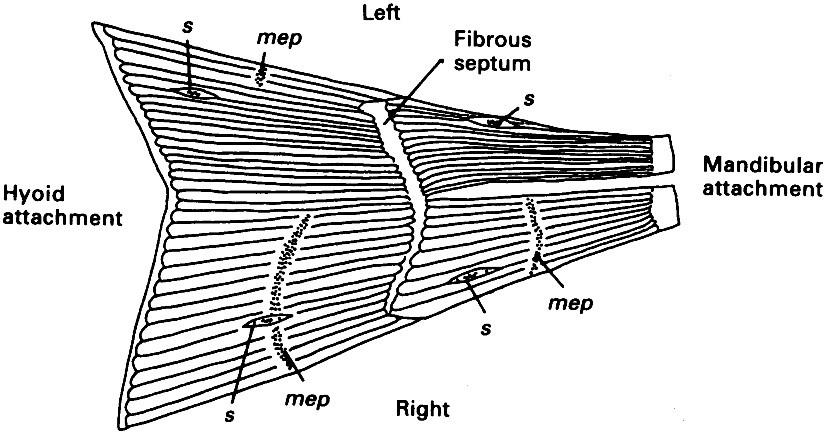


Fig. 16. Schematised tracing of a single frozen section traversing both geniohyoid muscles, fixed 14 days after section of the proximal XII nerve on the left side. Only innervated motor end plates (*mep*) are represented. The section contained 4 muscle spindles (*s*); on the left side, fusimotor innervation was absent but annulospiral endings were preserved.

Although muscle spindles were not observed in any of the experimental tongues, eight were identified in the posterior part of the tongue base, in five of the eight control tongues sectioned in the coronal plane. The positions of the spindles are shown in Figure 11. Each of the spindles was detected in at least 10 successive sections and their identity was not in doubt (Figs. 12, 13, 15).

Geniohyoid muscle

As noted by Lakers & Herring (1987), the geniohyoid muscle of the rat is partitioned almost completely by a fibrous septum. In coronal sections, 1–3 neuromuscular spindles were consistently observed in both the anterior (mandibular) and the posterior (hyoid) muscle belly (Fig. 14).

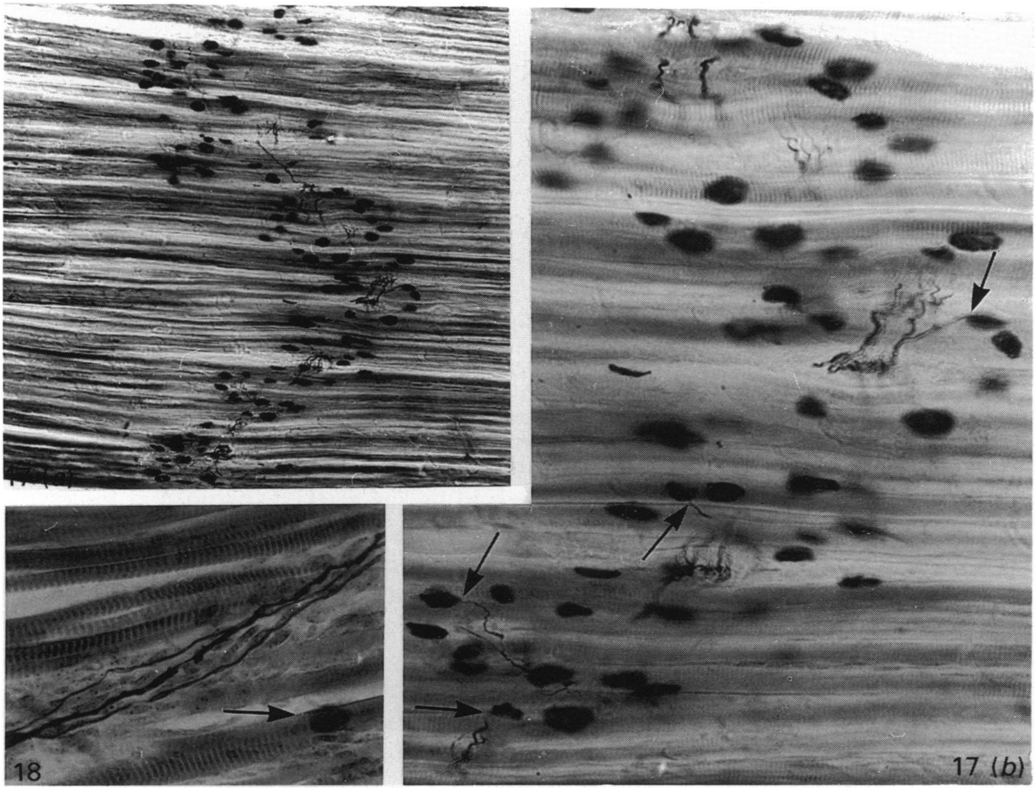
Distal XII nerve section eliminated all myelinated axons ipsilaterally. Following XII nerve section proximal to the ansa connection, three distinct sets of myelinated axons persisted within the muscle as follows (Figs. 16–19): (*a*) A small number (about 100) of motor end plates retained their innervation in the lateral part of the hyoid belly. In sagittal sections, these innervated plates extended through the full thickness of the muscle. (*b*) All of the neuromuscular spindles retained their afferent innervation whereas the fusimotor supply was lost. The afferent supply consisted of a single annulospiral nerve ending at the spindle equator. (*c*) Finely myelinated axons gave rise to unmyelinated terminals in the connective tissue, including the adventitial connective tissue of arterial walls.

Fig. 12(*a–b*). (*a*) Coronal section, left side of posterior tongue (normal rat), in the interval between mandible and hyoid bone. The arrow indicates position of a muscle spindle in genioglossus (*GG*). *GH*, geniohyoid; *HG*, hyoglossus; *HNL*, *HNM*, lateral, medial divisions of hypoglossal nerve; *LA*, lingual artery; *LI*, longitudinalis inferior; *ML*, midline regions; *SD*, salivary duct; *SG*, styloglossus; *TL*, transversus linguae. Paraffin-van Gieson. × 40. (*b*) Enlargement from Fig. 12(*a*), showing muscle spindle (arrow) in genioglossus. × 150.

Fig. 13(*a–b*). (*a*) Coronal section, right side of posterior tongue (normal rat). Arrow indicates a muscle spindle in the lateral edge of hyoglossus (*HG*). Other notations as in Fig. 12(*a*). Paraffin-van Gieson. × 40. (*b*) Enlargement from Fig. 13(*a*), showing muscle spindle (arrow) in hyoglossus. × 150.

Fig. 14. Control side of operated rat, showing muscle spindle (arrow) in geniohyoid. Protargol. × 80.

Fig. 15. Normal rat, showing muscle spindle (arrow) in longitudinalis inferior. The blood vessel (*bv*) turned upwards in a nearby section. Other notations as in Fig. 13(*a*). Paraffin-van Gieson. × 110.



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17 (b)

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Fig. 17(*a–b*). (*a*) Sagittal section of geniohyoid, close to the outer edge of the hyoid belly, 14 days after section of the proximal XII trunk. All of the motor end plates are cholinesterase-reactive, but the number of motor nerve fibres is greatly reduced. Cholinesterase-silver. × 160. (*b*) Enlargement from Fig. 13(*a*). Arrows indicate motor end plates having persistent innervation. × 500.

Fig. 18. Field from an adjacent section, showing three healthy axons in a partly depleted nerve branch close to its point of entry. The arrow indicates a denervated motor end plate. × 500.

Fig. 19. Longitudinal section of neuromuscular spindle in geniohyoid, 2 days after proximal XII nerve section. The fusimotor innervation, together with the extrafusal supply to motor end plates (bottom right) had degenerated completely but the primary afferent supply (arrows) was preserved.

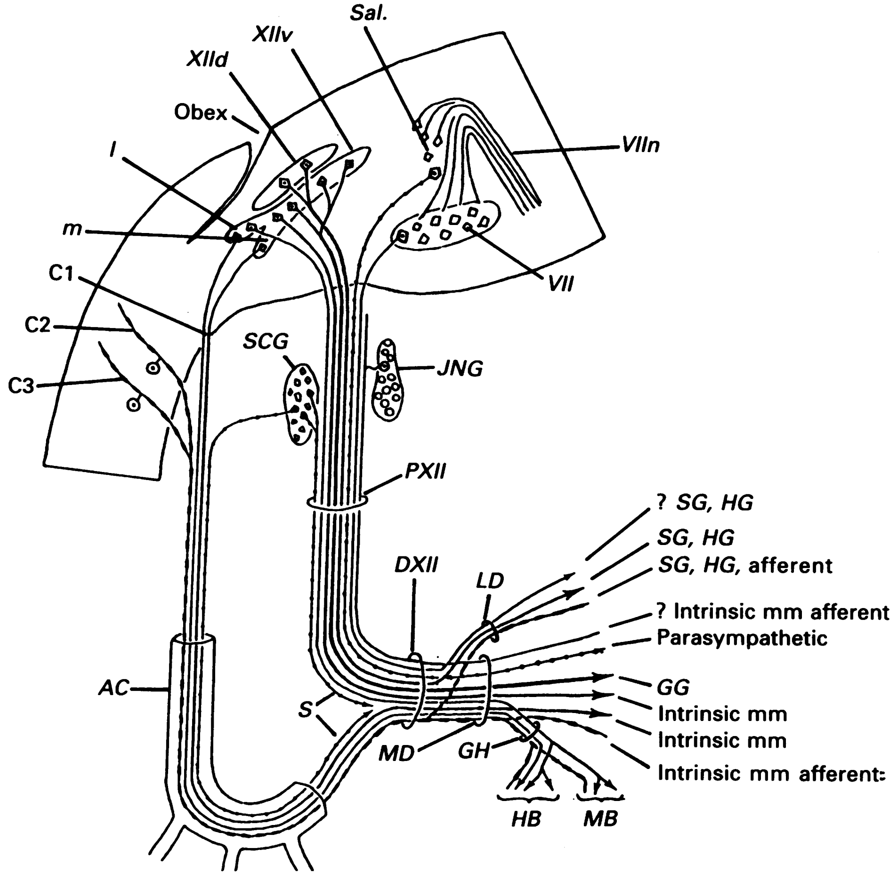

Fig. 20. Proposed fibre composition of the hypoglossal nerve. Hypoglossal nuclear elements: *XIId, v*, dorsal and ventral nuclear tiers; *l, m*, lateral and medial subnuclei of ventral tier. Other notations: *AC*, ansa cervicalis; C1, C2, C3, ventral rami of first three cervical nerves; *DXII*, distal *XII* trunk; *GG*, genioglossus; *GH*, geniohyoid branch; *HB*, hyoid belly of geniohyoid; *HG*, hyoglossus; *JNG*, jugulo-nodose ganglion; *LD*, lateral division of XII; *MB*, mandibular belly of geniohyoid; *MD*, medial division of XII; *PXII*, proximal XII trunk; *S*, sympathetic (further course not shown); *Sal.*, salivatory nucleus; *SCG*, superior cervical ganglion; *SG*, styloglossus; *VII*, facial nucleus; *VIIn*, facial nerve.

DISCUSSION

The fibre composition of the hypoglossal nerve in the rat has proved to be quite diverse. Figure 20 summarises the principal findings derived from the present study.

Taken together, the results of retrograde neuronal tracing and anterograde fibre degeneration indicate, firstly, that a small number of axons supplying *intrinsic lingual muscles* travel from the medial subnucleus of the ventral nuclear tier, by way of the ansa cervicalis, and reach the distal XII trunk for distribution. Indeed, Kitamura *et al.* (1985) proposed that these ventral tier neurons, because of their small soma size, should be destined for the intrinsic musculature. This was shown conclusively in our study.

Secondly, a small number of axons supplying the *geniohyoid muscle* travel from the lateral subdivision of the ventral nuclear tier by way of the ansa cervicalis and distal XII trunk. This finding confirms the second (1983, 1986) proposal of Kitamura *et al.* which was based on retrograde labelling following HRP application to the XII branch to the geniohyoid. The targets of these axons have proved to be extrafusal muscle fibres in the posterior (hyoid) belly of the muscle. The remaining extrafusal fibres (both bellies) and the intrafusal fibres of geniohyoid neuromuscular spindles, are supplied from the lateral subnucleus by axons travelling in the main XII trunk.

Thirdly, *proprioceptive afferents* from the tongue and geniohyoid leave the distal XII trunk and enter the ansa cervicalis to reach spinal ganglia C2 and C3. A small number leave the proximal XII trunk instead, to reach cell bodies in the jugulo-nodose ganglion complex. These findings are in general agreement with those of Neuhuber & Mysicka (1980) who estimated that about 200 cervical ganglion cells were labelled following HRP application to the distal XII trunk and about 16 in the jugulo-nodose ganglion. On the basis of HRP application to the medial or lateral divisions of the XII trunk, these workers concluded that the vagal neurons were destined entirely for intrinsic lingual muscles and the cervical neurons mainly for extrinsic lingual muscles and geniohyoid.

The occurrence of labelled XII-directed neurons within the branchial efferent component of the *facial* (*VII*) *nucleus* is not unique to the rat. Chibuzo & Cummings (1982) found labelled cells in the caudoventral pons in dogs following HRP injection into styloglossus, hyoglossus, or genioglossus. Satomi, Ninomiya, Abe & Takahishi (1985) described labelling within the ventromedial and ventrolateral subnuclei of VII in cats following HRP injection into the anterior half of the tongue. In the present study, VII nuclear labelling followed either dye application to the XII trunk or dye insertion into the tongue; it was unaffected by extracranial section of the VII nerve. The axons concerned are therefore considered to have descended from the caudal pons to join intramedullary rootlets issuing from the XII nucleus. It is of interest that Langman (1975) anticipated the possibility of a VII nerve motor contribution to the tongue on the grounds that some premuscle cells of hyoid arch origin might remain in the floor of the embryonic pharynx after migration of the main hyoid sheet onto the splanchnocranium. However, it is difficult to provide a wholly satisfactory explanation for the present findings, which imply the migration (neurobiotaxis) of hypoglossal neurons into the caudal end of the main VII nucleus during embryonic life.

Labelling of cells in the *salivatory nucleus* of the pons was initially surprising because, although the pontine rather than the medullary reticular formation is the source of preganglionic fibres supplying intralingual ganglia in the rat (Contreras, Gomez & Morgren, 1980; Yu & Srinivasan, 1980), the chorda tympani has been the only known vehicle for these fibres. This seems to be true for the cat, where all of the ganglia observed in the middle and anterior parts of the tongue are suspended from branches of the lingual nerve, which distributes the chorda tympani within the tongue (FitzGerald & Alexander, 1969). In the rat, however, a minority of the ganglia are clearly attached for the observed labelling in the reticular formation following dye insertion into the tongue or application to the XII trunk. Further investigation is warranted, in order to determine what species differences may exist. Intralingual ganglia are present in a wide range of vertebrates; their vasodilator function has been accounted for by co-liberation of vasoactive intestinal polypeptide onto arterial walls (Baecker, Yanaihara & Forssmann, 1983; Pleschka, 1988).

Retrograde labelling of several cells in the *jugulo-nodose ganglion* confirms the work of Neuhuber & Mysicka (1980), who identified HRP-labelled cells in the same location. These authors noticed preferential labelling after HRP application to the medial division of the XII nerve, and considered the genioglossus to be a likely target for the afferent neurons concerned. In the cat, the X–XII connection contains fibres responsive to passive stretching of the tongue (Zapata & Torrealba, 1971), but the exact peripheral and central destinations of the vagal contribution to XII are unknown. The proximal end of the XII nerve seems to be devoid of afferent fibres (Lodge, Duggan, Biscoe & Caddy, 1973).

The observed labelling of about a dozen cells in the *superior cervical sympathetic ganglion* was expected in view of the very fine linkages between the ganglion and the adjacent XII nerve in the upper part of the neck. Detailed dissections by Hedger & Webber (1976) indicate that sympathetic postganglionic fibres have in fact three possible routes of access to the tongue: via the XII trunk, via the ansa cervicalis, and in a perivascular network surrounding the lingual artery. In the present material a rich perivascular network of unmyelinated axons accompanied the artery into the tongue; although it received branches from the medial division of XII after entry, the network was not noticeably depleted by XII nerve section.

Finally, the occurrence of *neuromuscular spindles* in the base of the tongue deserves special mention. They were observed in five out of eight tongues examined in coronal paraffin sections. In addition to six observed in the longitudinalis inferior at the site mentioned by Smith (1989), one was seen in the hyoglossus and one in genioglossus. A much larger and more complete series will be required in order to discover their true incidence and the full range of possible locations. The apparent restriction of spindles to the base of the tongue warrants histochemical surveillance of this region. Tonic contraction of muscle fibres across the entire tongue base could provide necessary stability for movements of the dorsum and tip; muscle spindles are known to have a predilection for tonically active muscle fasciculi, rich in oxidative enzymes (Botterman, Binder & Stuart, 1978). Further analysis of spindle distribution in the tongue of the rat, and possibly in other laboratory mammals, may permit their source(s) of innervation to be determined. In monkeys, where spindles are relatively numerous in the intrinsic and extrinsic muscles, the XII nerve is fusimotor, and cervical dorsal root ganglia provide the afferent nerve supply (FitzGerald & Sachithanandan, 1979).

SUMMARY

The fibre composition of the hypoglossal nerve of the rat has been investigated by means of (*a*) retrograde neuronal labelling following application of fluorescent dyes to the XII nerve and/or to the ansa cervicalis, lingual nerve and facial nerve; and (*b*) anterograde fibre degeneration in silver-impregnated sections of the tongue following interruption of one or more of these nerves. A search for neuromuscular spindles was carried out in coronal paraffin sections of eight control tongues.

The ansa cervicalis was found to make three significant contributions to the distal XII trunk: one set of axons originated in the medial subnucleus of the ventral tier of the XII nucleus and contributed to the motor innervation of intrinsic lingual muscles: a second set originated in the lateral subnucleus of the ventral tier and contributed to the motor innervation of the posterior part of the geniohyoid muscle. A third set, having cell bodies in the two uppermost cervical ganglia, provided proprioceptive afferents to the tongue and geniohyoid.

A small number of somata in the caudal end of the facial nucleus contributed axons to the XII nerve prior to its emergence from the medulla oblongata. The salivatory nucleus (pons) also contributed pre-emergence fibres, which supplied autonomic ganglia destined for the supply of small arteries in the tongue.

The fibre composition of the XII trunk was completed by an afferent contribution from the jugulo-nodose ganglion complex of the vagus nerve, and by sympathetic fibres from the superior cervical ganglion.

In control material, eight neuromuscular spindles were detected in five out of eight tongues. Six occupied the longitudinalis inferior, one the hyoglossus, and one the genioglossus. All eight were close to the surface of the tongue base.

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