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The composition and central projections of the internal auricular nerves of the dog

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ABSTRACT

The cranial components and central terminations of the sensory nerves supplying the concave surface of the puppy's pinna, namely, the rostral, middle and caudal internal auricular nerves (RIAN, MIAN and CIAN) were investigated using horseradish peroxidase retrograde and transganglionic labelling techniques. All the 3 internal auricular nerves received contributions from the vagus. The RIAN received additional fibres from the trigeminal nerve while the MIAN and CIAN contained fibres derived from the facial nerves. In the brainstem, collaterals from the descending fibres of the afferents were given off at all levels to the medially located spinal trigeminal nucleus (SpV) which extended rostocaudally from the principal nucleus of the trigeminal nerve, subnuclei oralis, interpolaris and caudalis of the SpV to C1 or C2 cervical segment. The greatest density of central projections was observed in the subnucleus caudalis and C1. In the latter, the terminal field in the dorsal horn was roughly wedge-shaped, tapering off medially from lamina I towards lamina V. A somatotopic organisation was observed in the spinal trigeminal tract (SpVtr) and spinal trigeminal nucleus (SpV) in which the projection fibres and terminal fields of the RIAN were located lateral to those of the MIAN and CIAN. Some nontrigeminal nuclei, e.g. paratrigeminal and cuneate nuclei, nucleus X and the nucleus of the solitary tract were also labelled following HRP application to the internal auricular nerves. The localisation of the central projections of the internal auricular nerves as well as the 2nd order neurons in some specific nuclei to which the afferent fibres project is consistent with the concept of a brainstem somatovisceral link.

Key words: Spinal trigeminal nucleus and tracts; somatovisceral reflexes.

INTRODUCTION

The concave surface of the pinna of ear has been described as receiving a multiple nerve supply. Whalen & Kitchell (1983*a*, *b*) reported that most of the concave surface of the canine ear is supplied by the sensory branch of the facial nerve, whereas the rostral and caudal margins of the pinna are supplied by the auriculotemporal nerve and the great auricular nerve, respectively. Weddell et al. (1955) showed that the auricular branch of the vagus and the trigeminal nerve also contribute to the sensory innervation of the rabbit ear. The auricular branch of the vagus nerve leaves the nerve at the level of the superior ganglion and travels laterally to join the facial nerve which also innervates the concave surface of the pinna in man (Barr & Kiernan, 1993). It would appear from a literature review that except for the auriculotemporal nerve in the cat and rat (Beckstead & Norgren, 1979; Panneton & Burton, 1981; Contreras et al. 1982; Jacquin et al. 1982, 1983; Shigenaga et al. 1986*a*, *b*; Takemura et al. 1987), the central projections of the auricular branches of the above-mentioned cranial nerves have not been fully documented. The present study was therefore undertaken to examine the central projections of the auricular nerves (the terminology used in this text follows that of the Nomina Anatomica Veterinaria, 3rd edn, 1983) in the dog as well as to

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ascertain the extent of the contribution of afferent fibres from the various cranial nerves mentioned above.

MATERIALS AND METHODS

Surgical procedure and HRP application

Twenty-three puppies of either sex, weighing 1.8–3.2 kg, were used. Preanaesthetic treatment with atropine sulphate (0.02–0.04 mg/kg, subcutaneously) and diazepam (0.2–0.6 mg/kg, intravenously) was given 30 min before surgery. All surgical procedures were performed under general anaesthesia (sodium pentobarbitone, 22 mg/kg, intravenously). 4 μl of 40% free based horseradish peroxidase (HRP) (Sigma type VI, lot no. 121H9540, in 2% diethyl sulphoxide) combined with 2.0 μl of 0.25% cholera toxin subunit B conjugated with HRP (Sigma, lot no. 72H0752) were applied to the nerves as follows. The rostral, middle or caudal internal auricular nerves (RIAN, MIAN and CIAN, n = 9, 9 and 5, respectively, Fig. 1) were isolated and crushed. Each nerve was then inserted into a piece of polyethylene tube which had been slit longitudinally and the nerve had been securely enclosed in the tube filled with gelfoam sterile sponge (Upjohn, USA) soaked with the tracers. Both ends of the tube were then sealed with vaseline. The nerve was placed in situ for 1 h, after which it was severed and left in place for another hour. After that, the HRP-soaked gelfoam was removed and the nerve was rinsed repeatedly with sterile saline. The skin was closed with interrupted sutures.

Fig. 1. Schematic diagram showing the rostral (R), middle (M) and caudal (C) internal auricular nerves on the left lateral side of the head of a dog. The circles indicate the sites of HRP application for the respective nerves.

Table 1. *Number (mean ± S.D.) of labelled neurons in the ganglion associated with the corresponding cranial nerves following HRP application into the respective internal auricular nerves*

	V	VII	X*	C2	C3
RIAN (n = 9)	600 ± 160	—	9 ± 2	2	—
MIAN (n = 9)	—	21 ± 8	151 ± 83	—	—
CIAN (n = 5)	—	38 ± 26	137 ± 89	5 ± 3	1

Superior (jugular) ganglion of the vagus.

Fixation, sectioning and histochemistry

All puppies were allowed to survive for 48–96 h before they were killed. Animals killed between 72–84 h after the HRP application yielded the best results with optimal labelling. Each dog was given an overdose of sodium pentobarbitone before they were perfused intracardially with 0.5 l of warm heparinised Ringer's solution (containing 500 I.U./kg of heparin) followed by 2 l of freshly prepared fixative, consisting of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer (PB), pH 7.4 at 4 °C. This was followed by perfusion with 1 l of 10% sucrose in 0.1 M PB (pH 7.4) to remove any excess of fixative in the animal. After the perfusion of the brainstem and upper cervical cord (extending from the inferior colliculus to C3 segment), the trigeminal, geniculate, superior and inferior ganglia of both glossopharyngeal and vagus nerves together with the dorsal root ganglia of C1 to C3 and superior cervical ganglia were removed and stored overnight in PB containing 30% sucrose. 30 μm thick longitudinal sections of the respective ganglia and transverse sections of the brainstem and spinal cord were cut on a cryostat.

All sections from the ganglia and 1 in every 4 sections of the brainstem and spinal cord were collected serially in 0.1 M PB. They were reacted for HRP according to the tetramethylbenzidine (TMB, Sigma) technique of Mesulam (1978), and finally mounted on chrome alum, gelatin-coated slides. The sections were counterstained lightly with 1% neutral red and examined under the light microscope. Photomicrographs were taken either under bright or dark-field illumination.

Tissue analyses and cell counting

Under the light microscope, the HRP-labelled fibres appeared as linear arrays of closely packed granules, filled with reaction product, whereas the terminal fields appeared as irregular clusters of scattered fine granules. These distributions were plotted on a sheet of paper with the aid of a drawing tube attached to the microscope. For quantitative studies of HRP-labelled ganglia, only those neurons that showed a distinct outline as well as a clear nuclear zone and a homogeneous dark blue cytoplasmic labelling were

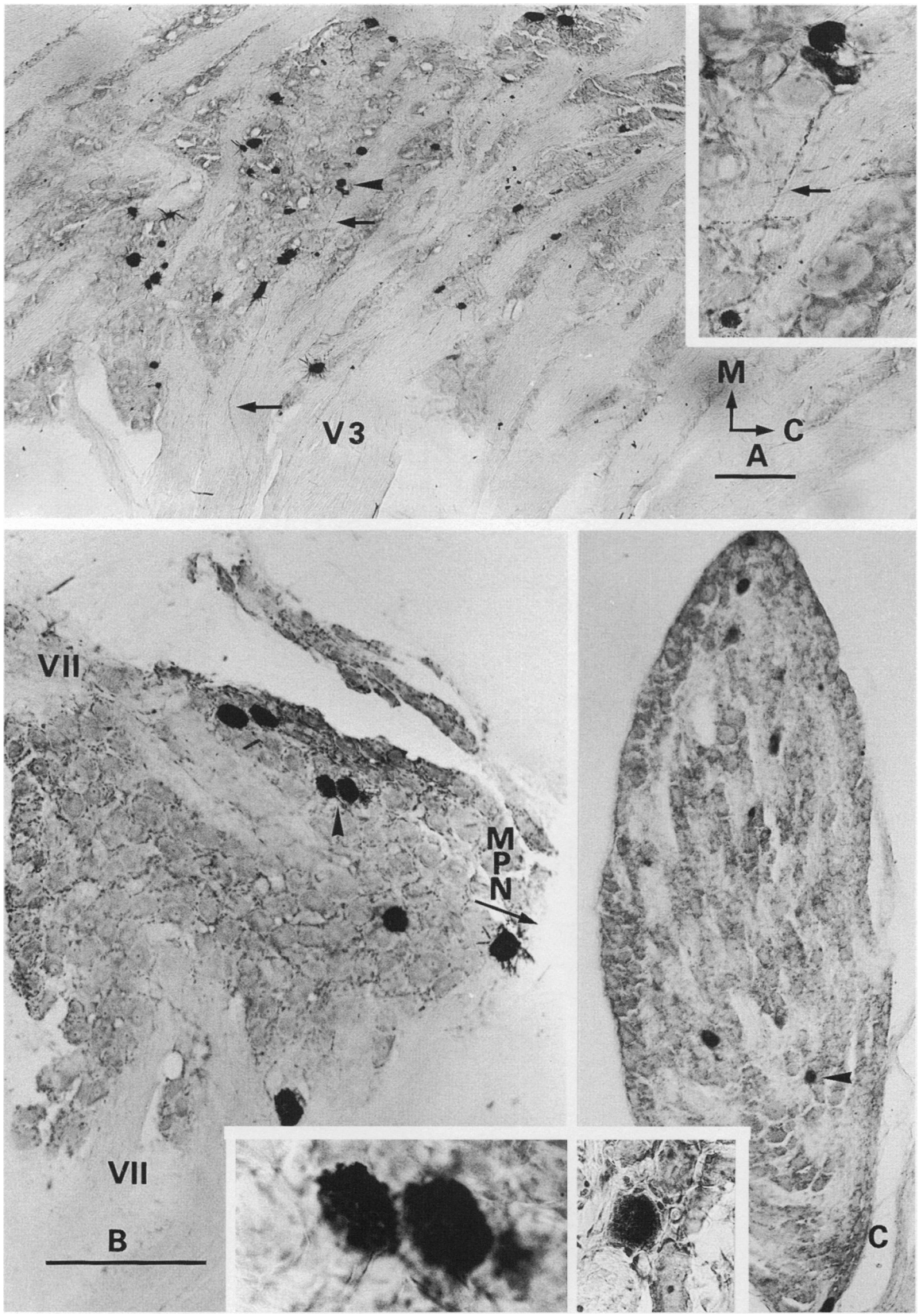

Fig. 2. Brightfield photomicrographs showing HRP-labelled neurons in the trigeminal (*A*), geniculate (*B*), and superior ganglia of the vagus nerve (*C*). The tracer was applied to the rostral, caudal and middle internal auricular nerves, respectively. Insets are higher magnification of the labelled neurons (arrowheads) and nerve fibres (arrows). M, medial; C, caudal; V3, mandibular nerve; VII, facial nerve; MPN, major petrosal nerve. Bar, 300 μm.

selected for measurement. The crude cell counts obtained in each of the ganglia were corrected using Abercrombie's method (1946).

RESULTS

Injection of HRP into the rostral internal auricular nerve (RIAN)

Labelled neurons in ganglia

Following HRP application to the RIAN, many labelled neurons were observed in the ipsilateral trigeminal ganglion (mean = 600 ± 160) (Table 1) but only occasionally in the superior ganglion of the vagus nerve and C2 dorsal root ganglion. In contrast, there were no labelled cells in the geniculate, superior cervical and C3 dorsal root ganglia. In the trigeminal ganglion, the labelled cells were distributed preferentially in the posterolateral region (Fig. 2*A*), including the prominent posterolateral protuberance. Here the labelled cells were distributed along its entire dorsoventral extent but the majority of them tended to be concentrated in the ventrolateral area. Labelled nerve fibres were also observed in the medial third of the mandibular branch and the lateral third of the sensory root of the ganglion.

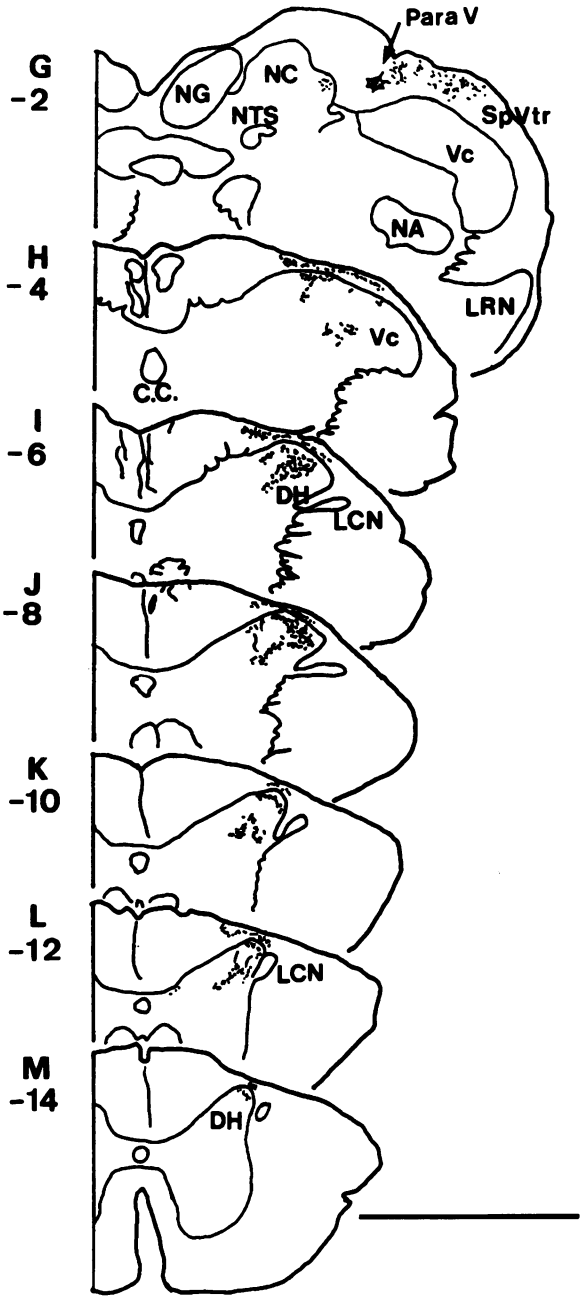

Fig. 3. For legend see opposite.

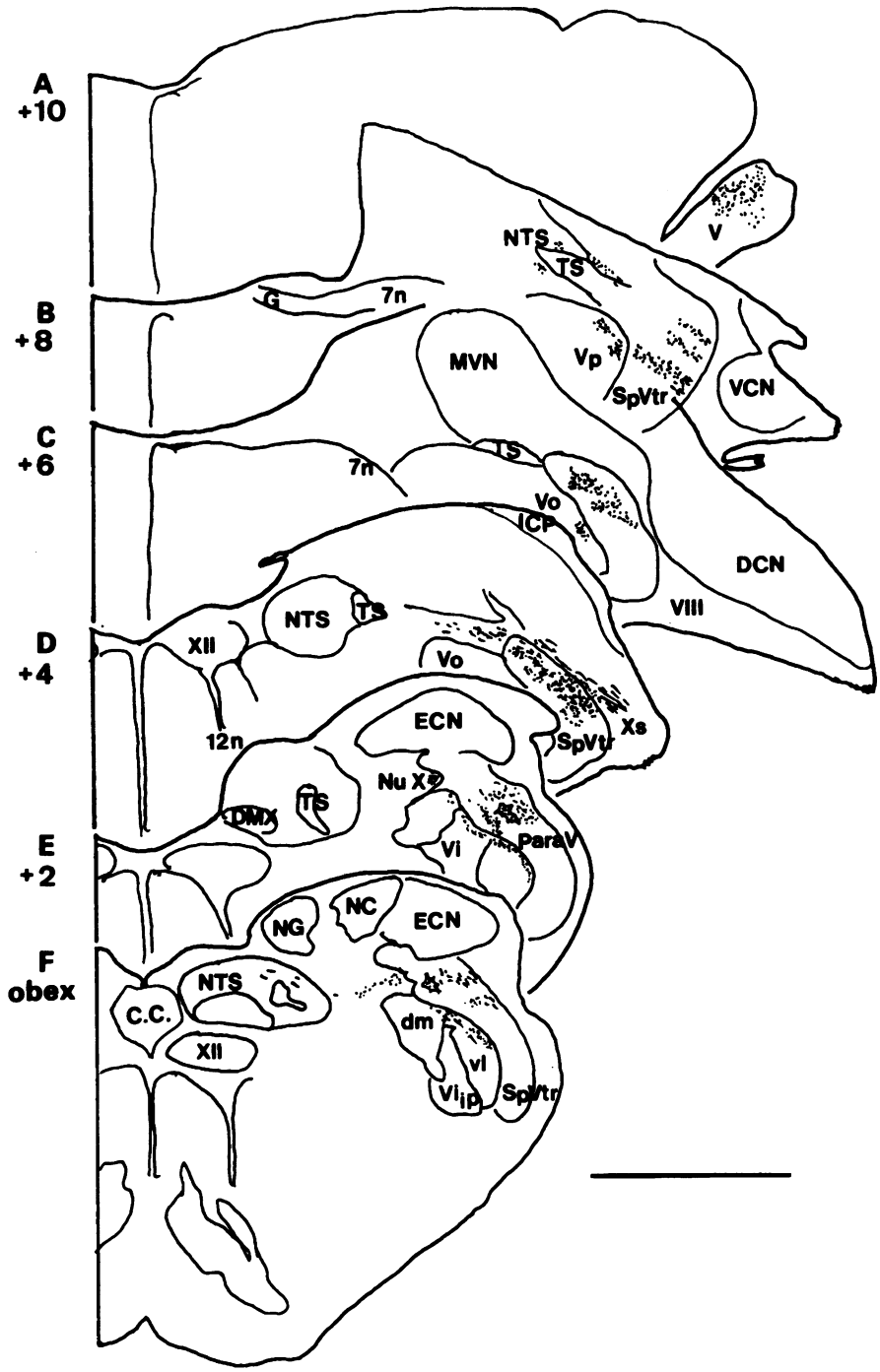

Fig. 3. Serial schematic diagrams from caudal pons to the 2nd cervical (C2) segment, showing the central distribution of the rostral internal auricular nerve. Stippled dots indicate HRP-labelled terminals while the interrupted lines represent HRP-positive fibres. The numbers on the left of the diagrams indicate the approximate distance in mm from the obex to facilitate comparison with the corresponding levels in Figures 6 and 7. Abbreviations (and for Figs 5–8): CC, central canal; DCN, dorsal cochlear nucleus; dm, dorsomedial region of subnucleus interpolaris of SpV; DH, dorsal horn; DMX, dorsal motor nucleus of vagus; ECN, external cuneate nucleus; G, genu of facial nerve; ICP, inferior cerebellar peduncle; ip, intermediate plexus of subnucleus interpolaris of SpV; LCN, lateral cervical nucleus; LRN, lateral reticular nucleus; MVN, medial vestibular nucleus; NA, nucleus ambiguus; NC, nucleus cuneatus; NG, nucleus gracilis; NTS, nucleus tractus solitarius; NuX, nucleus X; Para V, paratrigeminal nucleus; SpVtr, spinal trigeminal tract; TS, tractus solitarius; V, sensory root of trigeminal nerve; VCN, ventral cochlear nucleus; Vc, subnucleus caudalis of SpV; Vi, subnucleus interpolaris of SpV; vl, ventrolateral region of subnucleus interpolaris of SpV; Vo, subnucleus oralis of SpV; Vp, principal nucleus of trigeminal nerve; VII, facial nerve; VIIi, nervus intermedius of facial nerve; VIII, cochlear/vestibular nerve; XII, hypoglossal nucleus; Xs, sensory root of vagus nerve; 7n, facial nerve root, descending; 12n, hypoglossal nerve root.

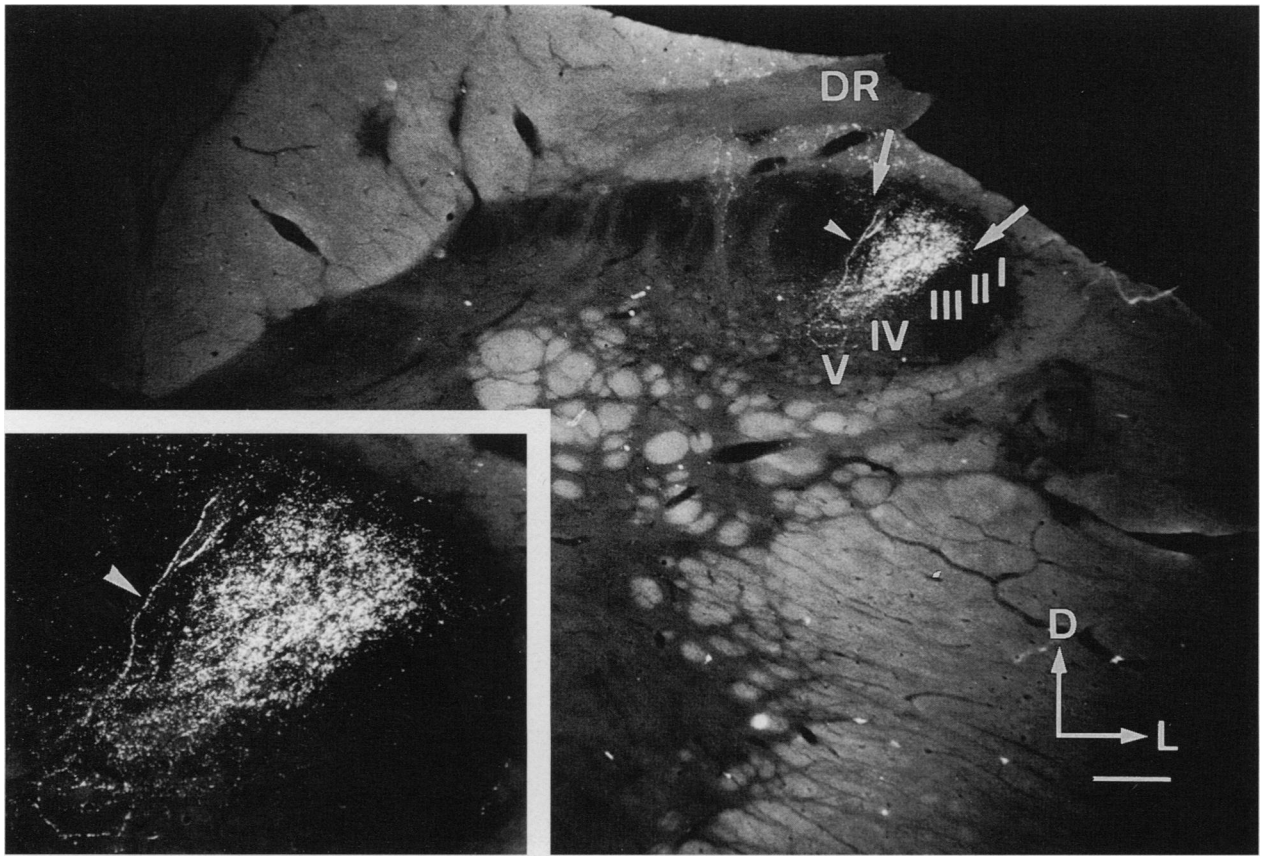

Fig. 4. Darkfield photomicrographs taken from transverse sections at the levels between the caudal subnucleus caudalis and rostral C1, showing wedge-shaped terminal fields (large arrows) following HRP application to the rostral internal auricular nerve. Terminal fields in the medullary dorsal horn occupy their lateral 1/2–2/3 territories. Note that the dorsal rootlets (DR) are devoid of HRP-positive fibres. Inset is a higher magnification of wedge-shaped terminal fields. Arrowheads point to the coarse projecting fibres. D, dorsal; L, lateral. Bar, 300 μm. Roman numbers I–V denote Rexed layers.

Central projections

Projections of RIAN caudal to the obex (Figs 3*G–M*, 4, 5*A*, *B*; Table 2). After application of free HRP combined with CTb-HRP to the RIAN, intense transganglionic labelling in the ipsilateral spinal trigeminal tract (SpVtr) and its neighbouring spinal trigeminal nucleus (SpV) as well as the dorsal horn of the cervical cord as far as the caudal level of the 2nd cervical (C2) segment (Table 2) was observed. In the SpVtr, the afferent fibres coursed caudally and occupied the dorsomedial half of this tract, whereas at the level of C1 and C2 cervical segments, the labelled fibres and terminals had shifted to the medial half of the tract (Fig. 3*J–M*). In the dorsal horn of C1 and C2, labelled fibres and terminals were distributed in a wedge-shaped terminal field. The terminal field was widest in laminae I and II, but tapered off towards laminae IV or V. A dense accumulation of HRP reaction product was usually observed in laminae II, III and IV (Fig. 4). A few coarse fibres (arrowheads, Fig. 4) in the superficial layer coursed for a considerable distance around the curvature of the terminal field before ending in the deeper laminae.

Besides projecting into the SpV and the adjacent dorsal horn, the central processes also terminated in the basal and dorsal extension of the paratrigeminal nucleus (Para V) in the SpVtr at the rostral and middle Vc (Figs 3*G*, 5*A*). Some projection terminals were also observed in the cuneate nucleus in the rostral Vc (Fig. 5*B*). HRP-positive fibres were also sparsely distributed in the dorsal and medial subnuclei of the nucleus tractus solitarius (Fig. 5*B*) at the junction of Vc and Vi.

Projections of the RIAN rostral to the obex (Fig. 3*A–F*, Table 3). At the level of subnucleus interpolaris (Fig. 3*E–F*) densely labelled fibres and terminals were localised in the dorsomedial half of SpVtr. The projection fibres and terminals appeared as a dense burst of labelling from SpVtr into the nearby subgroup of Vi, i.e. dorsomedial, ventrolateral, intermediate plexus and magnocellularis of caudalis (Vc.m). In the caudal Vi especially around the obex, the Para V and nucleus X (Mantles-St John & Tracey,

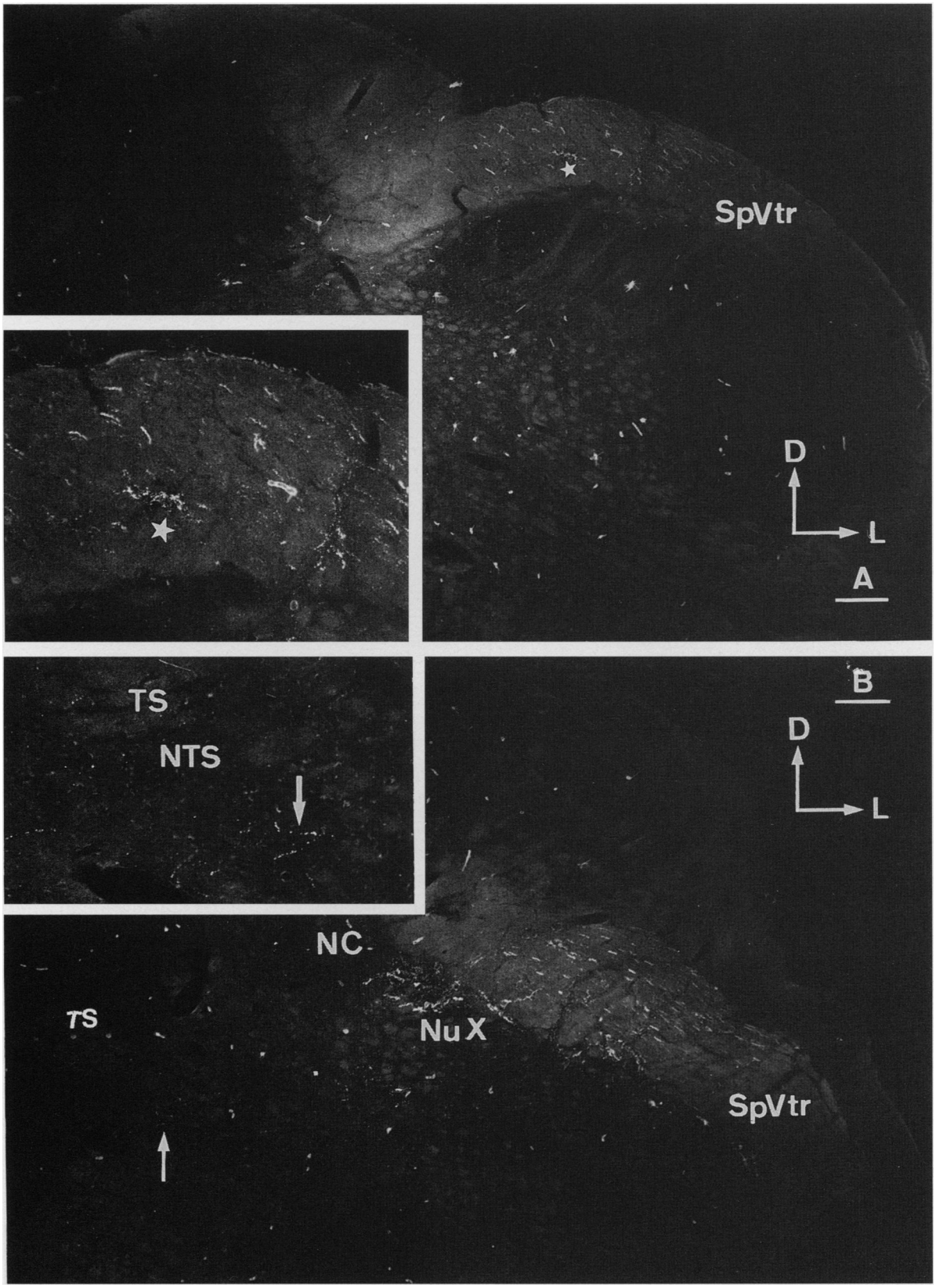

Fig. 5. Darkfield photomicrographs showing HRP-labelled terminals in some nontrigeminal nuclei when the tracer is applied to the rostral internal auricular nerve. (*A*) Paratrigeminal nucleus (Para V) (asterisk) at the level of subnucleus caudalis (see also inset). Some positive fibres are scattered in the spinal trigeminal tract (SpVtr) and these project caudally in the dorsal part of the SpVtr. At the level of the rostral subnucleus caudalis (B), HRP-positive terminal fibres can be traced to the ventrolateral part of the cuneate nucleus (NC), nucleus X (Nu. X) and few fibres terminate in the nucleus tractus solitarius (NTS) (arrows, see also inset). D, dorsal; L, lateral. Bar, 300 μm. Abbreviations as shown in Fig. 3.

Table 2. *Central projections of the internal auricular nerves to the subnucleus caudalis of SpV in the closed medulla and the dorsal horn of C1 and C2 segments**

	Rostral internal auricular nerve								
	V. caudalis			C1			C2		
	R	M	C	R	M	C	R	M	C
SpVtr	3+/3+	/3+	2+/3+	2+/2+	3+/2+	2+/2+	1+/1+	1+/	—
Para V	3+/	3+	—	—	—	—	—	—	—
Dorsal horn									
I, II	1+/1+	1+/1+	1+/1+	1+/1+	3+/1+	3+/1+	2+/1+	1+/1+	1+/
III, IV	—	—	1+/1+	1+/1+	3+/1+	3+/1+	2+/1+	2+/1+	—
V	—	1+/	1+/1+	2+/2+	3+/1+	3+/1+	2+/1+	2+/	
NC	2+/	—	—	—	—	—	—	—	—
NTS d.m.	1+/1+	—	—	—	—	—	—	—	—

	Middle internal auricular nerve					Caudal internal auricular nerve					
	V. caudalis			C1		V. Caudalis			C1		
	R	M	C	R	M	R	M	C	R	M	C
SpVtr	1+/1+	1+/1+	/1+	/1+		1+/1+	1+/1+	/1+	/1+	/1+	/1+
Para V	2+/	2+/	—	—	—	2+/	1+/	—	—	—	—
Dorsal horn											
I, II	—	—	—	2+/	2+/	1+/1+	1+/1+	1+/	1+/	3+1	1+/
III, IV	—	—	—	2+/	2+/	—	—	—	1+/	3+/	1+/
NC	1+/1+	—	—	—	—	2+/1+	—	—	—	—	—

* Numerals denote intensity of HRP-labelled terminals/fibres. 3+, very strong labelling; 2+, strong labelling; 1+, moderate labelling. R, rostral, M, middle, C, caudal.

1987) showed the densest HRP positive terminals (Fig. 5*B*); a few positive fibres were observed from the tip of the Vi towards the NTS (Fig. 3*F*). Very few HRP-filled terminals were present in the dorsal subnuclei of the NTS (Figs 3*F*, 5*B*). A small number of labelled terminals were also seen in the reticular formation dorsomedial to the caudal Vi.

At the level of subnuclei oralis and principal nucleus of the trigeminal nerve (Fig. 3*A–D*) most of the HRP-labelled fibres and terminals were located in the dorsolateral half of the SpVtr. The dorsomedial subgroup of caudal Vo received more labelled fibres and terminals than the other subdivisions. Sparsely labelled terminals appeared in the ventral subgroup of the caudal Vp only. The NTS also received HRP-labelled fibres and terminals at some levels. A few HRP-labelled fibres appeared in the lateral and ventral NTS at the level of caudal Vo. However, at the middle level of Vp some HRP-positive fibres and terminals passed immediately dorsal to the SpVtr, traversed around the solitary tract and consequently, terminated in the medial subnucleus of the NTS (Fig. 3*B*).

At the pontomedullary junction, i.e. at about the level of the middle Vp, the dorsolateral half of the sensory root of the trigeminal ganglion was densely populated with HRP-labelled axons. After entering the pons laterally, most fibres turned caudalward, and remained in the dorsal half of the SpVtr (Fig. 3*A*, *B*). The labelled vagal afferent fibres (Xs) to the lateral medulla (Fig. 3*D*) occurred approximately at the level of the junction of Vi and Vo and, on entry into the medulla, coursed immediately dorsal to the SpVtr either as single axons or small bundles.

Middle and caudal internal auricular nerve (MIAN and CIAN)

Labelled neurons

Following the application of HRP to the MIAN, labelled-neurons were observed mostly in the superior (jugular) ganglion of the vagus nerve (mean = 151 ± 83) and some in the geniculate ganglion of the facial nerve (mean = 21 ± 8). In the former instance, the HRP-labelled cell bodies were scattered randomly (Fig. 2*C*). In the latter instance, the HRP-labelled geniculate neurons were located near the origin of the major petrosal nerve (Fig. 2*B*, Table 1). HRP-positive fibres were also observed both in the peripheral and central processes of these ganglia. Labelled neurons

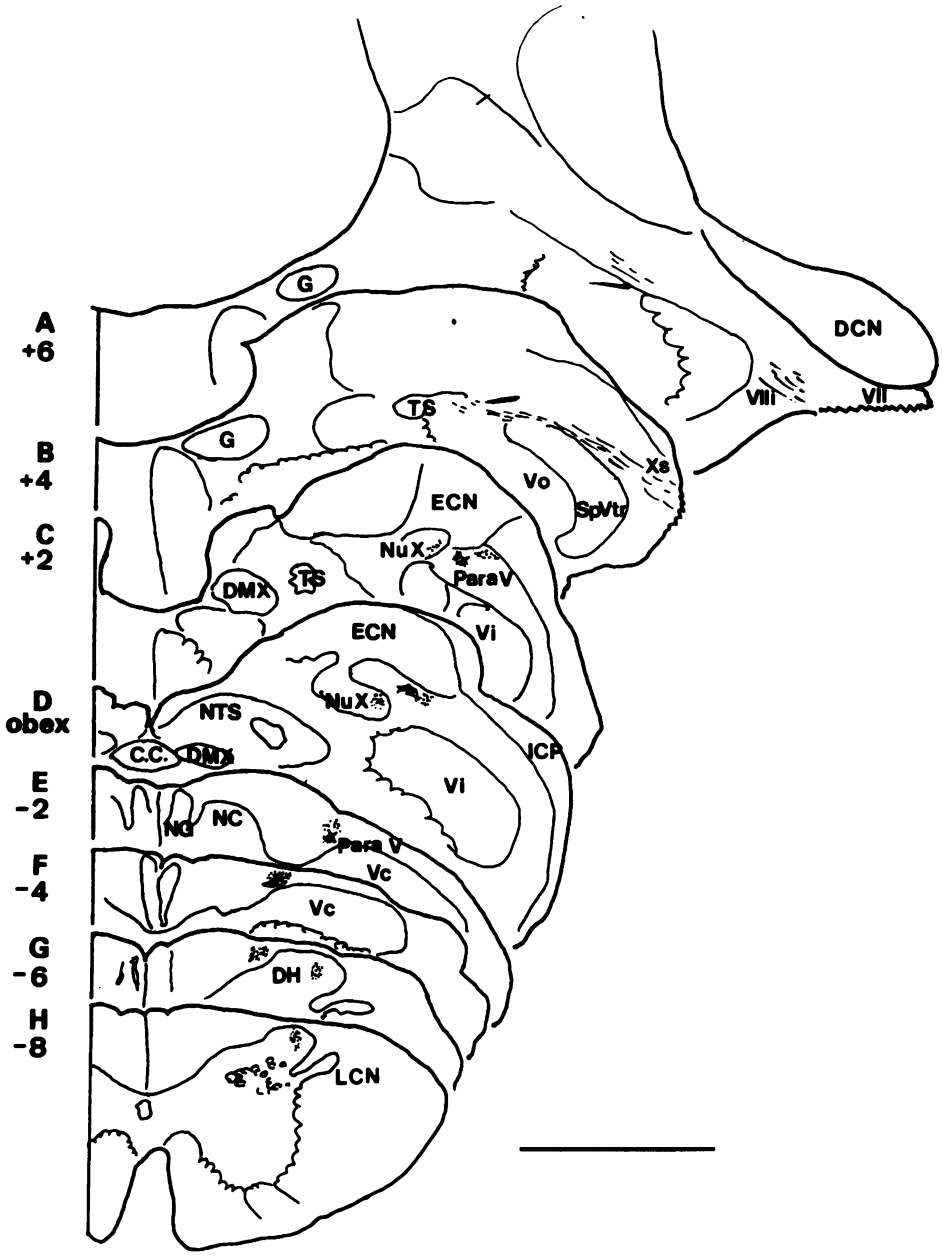

Fig. 6. Schematic diagram of serial transverse sections depicting the central distribution of labelled axons and terminals after the application of HRP to the middle internal auricular nerve.

were absent in the trigeminal, superior cervical and C2, C3 dorsal root ganglia.

Labelled cell bodies were also observed in the superior (jugular) ganglia of the vagus (mean = 137±89) and geniculate ganglion (mean = 38±26) following the application of HRP to the severed CIAN (Table 1). The distribution of the labelled neurons in the ganglia was similar to that after HRP application of the MIAN, i.e. in the geniculate ganglion, the HRP-labelled cells were located near the exit of the MPN, whereas in the superior ganglion, they were randomly distributed (Fig. 2*B*, *C*). HRP reaction product was also observed in the central and peripheral processes of the neurons in both ganglia. A few labelled neurons were observed in the C2 and C3 dorsal root ganglia. The trigeminal and superior cervical ganglia contained no HRP-labelled neurons.

Central projections

Following HRP application to the MIAN and CIAN, labelled afferent fibres and terminals were confined to the ipsilateral brainstem and C1 cervical segment (Figs 6, 7). The terminal fields of both nerves showed considerable overlap along the rostrocaudal extent of the SpVtr and they tended to be located more medially than those of the RIAN (Fig. 8).

Central projections caudal to the obex (Figs 6*E–H*,

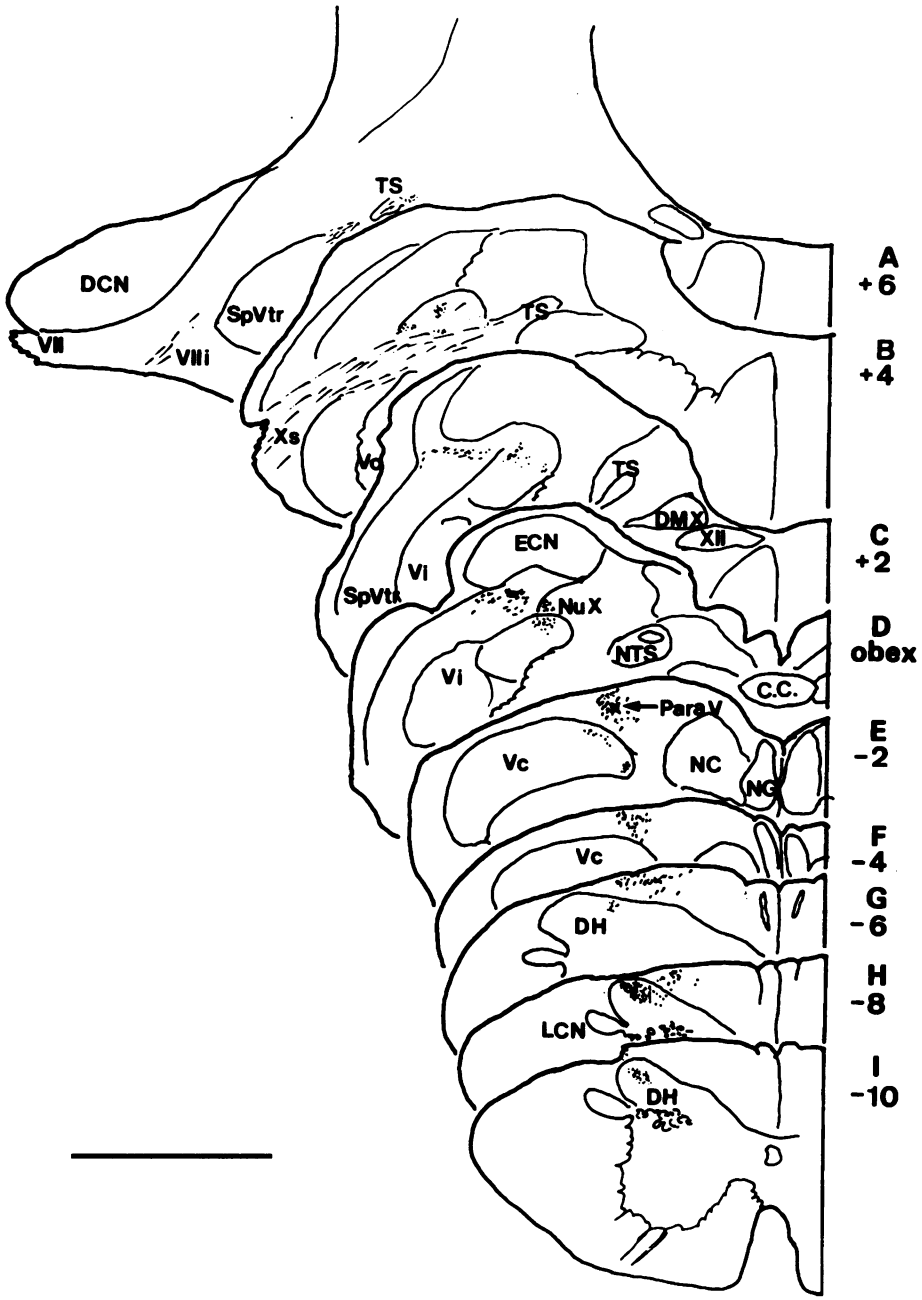

Fig. 7. Serial schematic diagrams of projected transverse sections depicting the central distribution of labelled axons and terminals after the application of HRP to the caudal internal auricular nerve. Abbreviations as shown in Fig. 3.

7*E–I*). HRP-labelled fibres and terminals from the CIAN extended more caudally than those of the MIAN to reach the C1 segment. Some fibres of CIAN penetrated laminae I to IV of the C1 dorsal horn. A complete wedge-shaped terminal field was observed only in the dorsal horn of the middle C1 segment (Fig. 7*H*), but not for fibres and terminals from the MIAN. The terminations were located in the rostral and middle C1 segment (Fig. 6*G–H*). At the level of Vc (Figs 6*E–F*, 7*E–F*), only the terminals and fibres of CIAN projected to the superficial laminae (lamina I and II). Besides the projections in the dorsal horn, a moderate number of terminals were also observed in the paratrigeminal and cuneate nuclei.

Central projections rostral to the obex (Figs 6*A–D*, 7*A–D*). In the spinal trigeminal tract rostral to the obex, only moderate to sparse HRP-positive fibres and terminals both from the MIAN and CIAN were observed either at the level of Vi or rostrally in the caudal Vo. At the caudal Vo, only a few HRP-labelled fibres were present (Table 3). The positive fibres were also observed to project rostocaudally in the dorsomedial part of the SpVtr, such fibres were more medially placed than those of the RIAN. The HRP-

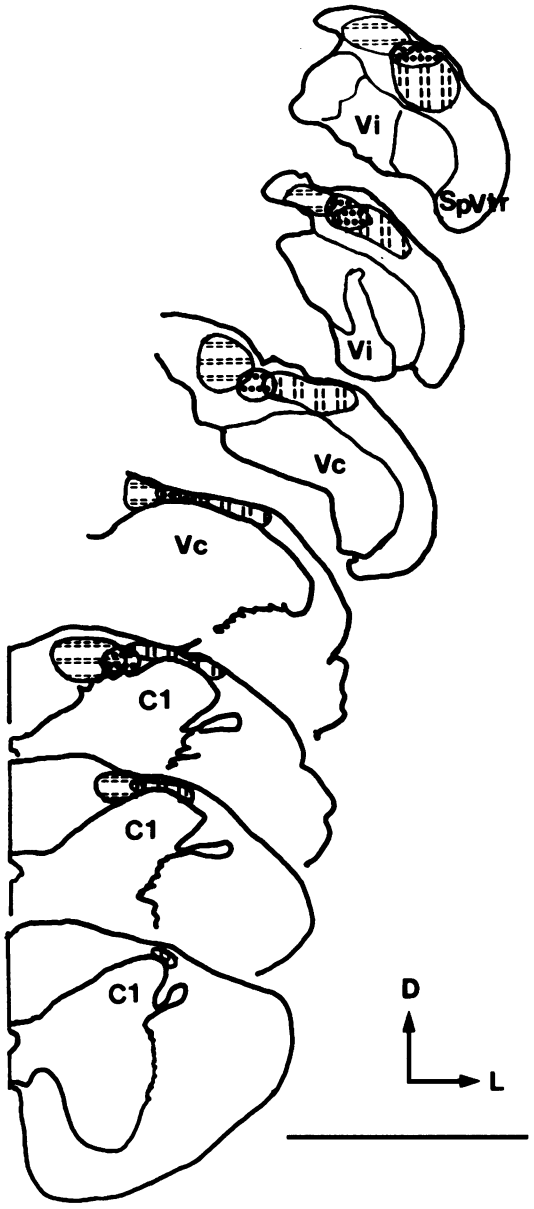

Fig. 8. Line drawings of transverse sections at different levels of the brainstem and cervical segments illustrating the territories of terminals and fibres from the internal auricular nerves. The areas delineated by vertical and horizontal lines represent projection territories of the rostral and caudal internal auricular nerves, respectively; the dotted areas represent projections of the middle internal auricular nerve. Abbreviations as shown in Fig. 3.

labelled vagal fibres entering the lateral medulla at the level of caudal Vo were often arranged into 2 fascicles (Figs 6*B*, 7*B*). The major fascicle followed a course over the dorsal edge of SpVtr and ascended to the dorsal, lateral and ventromedial subnuclei of the NTS while the smaller fascicle, which was located at the dorsal tip of the SpVtr, projected caudalward in the dorsal quadrant of the spinal tract (Figs 6*C–H*, 7*C–H*). Dense HRP-positive terminals from the MIAN were localised in the paratrigeminal nucleus at the level of Vi and nucleus X at the level of caudal Vi, respectively. The terminal distribution of CIAN followed that of MIAN at the level of Vi, but their terminals in addition projected to the dorsomedial subgroup of the Vi. At the rostralmost part of Vo, fibres from the nervus intermedius of the facial nerve (VIIi) penetrated the ventrolateral medulla immediately ventral to the VIIIth cranial nerve (Figs 6*A*, 7*A*). A few labelled fibres crossed the dorsal surface of the SpVtr and traversed obliquely to terminate in the lateral and ventromedial subnuclei of the nucleus tractus solitarius.

Table 4 summarises the central projections of the respective internal auricular nerves in the entire rostrocaudal extent of the brainstem extending from the level of the inferior colliculus to C2 spinal cord segment.

DISCUSSION

Three sensory nerves have been described as supplying the skin of the concave surface of the canine concha (Fig. 1). Despite the inconsistency in their nomenclature (Ammann et al. 1978; Whalen & Kitchell, 1983*a*, *b*; Evans & Kitchell, 1993), the terms rostral, middle and caudal internal auricular nerves adopted in the excellent text of Miller's Anatomy of the Dog (Evans, 1993) have been generally accepted in our study. The rostral internal auricular nerve (RIAN) has always been regarded as a terminal branch of the auriculotemporal branch of the trigeminal nerve, supplying the rostral auricular border both on its concave and convex aspects. The results of the present study have clearly demonstrated that the major component fibres of the RIAN originate from the trigeminal nerve, with a minor contribution from the vagus and the 2nd segment of the cervical spinal cord. A cutaneous branch from the facial nerve was found to innervate the concave surface of the pinna of the dog as judged electrophysiologically and from behavioural changes (Whalen & Kitchell, 1983*b*). Evans & Kitchell (1993) therefore reached the conclusion that the middle and caudal internal auricular nerves (MIAN and CIAN) are cutaneous branches of the facial nerve. By means of dissection, Weddell et al. (1955) had previously reported that the vagus nerve supplied the convex surface of the rabbit ear rather than the concave surface. From the present retrograde labelled study, the majority of fibres in the MIAN and CIAN are vagally derived, i.e. about 88% and 79% of the neurons of their associated ganglion were labelled, respectively.

No labelled neurons were observed either in the superior or inferior ganglia of the glossopharyngeal

Table 3. *Central projections (labelled terminals/labelled fibres) of the internal auricular nerves in the brainstem rostral to the obex**

	Rostral internal auricular n.			Middle internal auricular n.			Caudal internal auricular n.		
	Caudal	Middle	Rostral	Caudal	Middle	Rostral	Caudal	Middle	Rostral
V. interpolaris									
dm	2+/1+	2+1+	1+/1+	—	1+/	1+/	—	2+/	1+/
Int. plexus	1+/1+	1+/1+	1+/1+	—	—	—	—	—	—
vl	2+/1+	1+/1+	1+/1+	—	—	—	—	—	—
SpVtr	3+/3+	2+/2+	2+/1+	1+/1+	1+/1+	1+/1+	2+/2+	2+/2+	2+/2+
Para V	3+/	1+/	—	3+/	1+/	—	3+/	1+/	—
Cm	3+/1+	—	—	—	—	—	—	—	—
NTS d.	1+/1+	—	—	—	—	—	—	—	/2+
Nu. X.	3+/	—	—	2+/	—	—	1+/	—	—
V. oralis									
vl.	—	2+/2+	—	—	—	—	—	—	—
SpVtr	3+/3+	2+/2+	2+/2+	/1+	—	—	/2+	—	—
NTS v.	/1+	—	—	—	1+/1+	1+/1+	—	1+/1+	1+/1+
V. principalis									
v.	1+/	—	—	—	—	—	—	—	—
SpVtr	2+/2+	2+/2+	—	—	—	—	—	—	—
NTS m.	—	2+/2+	—	—	—	—	—	—	—

* Numerals denote intensity and number of labelled elements as in Table 2.

Table 4. *Summary of the central projection (+) of the internal auricular nerves*

	Vp	Vo	Vi	**Vc**	**C1**	**C2**	Para V	NC	NTS	Nu. X
RIAN	+	+	+	+	+	+	+	+	+	+
MIAN			+		+		+	+	+	+
CIAN			+	+	+		+	+	+	+

* Bold lettering represents large concentrations of labelling of terminal fields.

nerve after HRP application to the auricular nerves. This suggests that the 9th cranial nerve does not supply the auricle. A small number of HRP-positive neurons were observed in the 2nd and 3rd cervical dorsal root ganglia after HRP application to the RIAN and CIAN. On the other hand, HRP-positive fibres were not detected in the upper cervical dorsal rootlets. It is therefore speculated that such labelling could be due to some communicating fibres between the upper cervical spinal nerves and the internal auricular nerves. When the fluorescent dyes, Fast Blue and Diamidino Yellow were injected into the skin of the ear of rats in which the external or internal carotid nerves were intact, Flett & Bell (1991) showed that some neurons in the superior cervical ganglion (SCG) fluoresced. They concluded that the fluorescent labelled neurons must be vasomotor in function. In the present investigation using HRP, labelled neurons were never observed in SCG and this precludes the argument of tracer diffusion or leakage from the application site.

The results of the present study therefore indicate that the vagus, trigeminal and facial nerves all contribute general somatic afferents to the surface of the pinna in the dog, with minor contributions from the C2 and 3 spinal nerves.

Taren (1964) reported that the cutaneous components of the facial, glossopharyngeal and vagus nerves in the monkey are admixed with the mandibular division of the trigeminal nerve in its dorsomedial location in the spinal trigeminal tract. Furthermore, the central processes of the 3 divisions of the trigeminal nerve terminated somatotopically in the SpV and SpVtr, so that the fibres from the mandibular division occupied the dorsomedial angle of the tract. Such a somatotopic pattern has been confirmed by transganglionic transport of HRP applied to the individual sensory branches of the mandibular division, including the auriculotemporal nerve (Panneton & Burton, 1981; Shigenaga, 1986*a*, *b*; Takemura et al. 1987). The present results are in general agreement with these studies and, in addition, have shown that the terminal fields of the CIAN and MIAN in the SpVtr (Fig. 8) are located more medially than that of the RIAN.

Stover et al. (1992) demonstrated by the degeneration method that the largest number of the trigeminal afferents projected to the rostral two-thirds of the Vc and then descended caudally to the level of C2. The present results with HRP transganglionic transport are in agreement with their study, showing that the majority of the central processes of the IANs projected

and terminated in the rostral two-thirds of the Vc. Abrahams et al. (1984) who applied HRP to the C2 and C3 dorsal rami of the rat localised the reaction product throughout the entire length of NC and dorsal rootlets. In the present study, HRP-positive terminals were confined to the ventrolateral part of the NC at the level of Vi caudal to the obex and at no time were positive fibres observed in the upper cervical rootlets. The present results therefore suggest that the central processes of IANs are distributed via the cranial nerves rather than the cervical nerves.

The present results showed that the afferents of IANs project not only to the Vc and upper cervical segments, but also to Vi, Vo and Vp. The Vp is a homologue of the dorsal column nuclei and its ventral part projects to the posterior medial ventral nucleus of the thalamus (Panneton & Burton, 1981; Yasui et al. 1983; Mantles-St John & Tracey, 1987). This subnucleus may therefore subserve the discriminatory aspects of touch applied to the auricular region of the skin. The functional significance of Vo is still not clear, although Jacquin & Rhoades (1990) suggested that both Vo and Vi could be involved in sensory discrimination. The caudal portion of the SpV, especially the subnucleus caudalis (Vc) including the upper cervical dorsal horn, has been considered to be the major site for the relay of nociceptive information and to be implicated in trigeminal somatosympathetic reflex function (Chudler et al. 1991; Yonehara et al., 1992; Bereiter et al. 1994; McHaffie et al. 1994).

The present study has shown that at the level of Vi, several nontrigeminal nuclei including the cuneate nucleus (NC), nucleus X, Para V and NTS receive central projections from the IANs. NC has been reported to receive trigeminal and vagal sensory projections at its ventrolateral part (Kerr, 1962; Rhoton et al. 1966; Beckstead & Norgren, 1979; Jacquin et al. 1982, 1983). The projection of IANs to NC is thought to be involved in touch and proprioception from the pinna (Rhoton et al. 1966). Furthermore, it has been shown by single neuron recording in the rat that the nucleus X (Nu X) receives cutaneous inputs from the outer ear and periaural region, and that its efferent fibres project to the cerebellum and thalamus (Campbell et al. 1974; Mantles-St John & Tracey, 1987). Different cranial nerves, e.g. the trigeminal, the nervus intermedius of the facial and the vagal sensory nerve, have also been found to project to the Para V (Rhoton et al. 1966; Beckstead & Norgren, 1979; Contreras et al. 1982; Shigenaga, 1986*a*, *b*; Pfaller & Arvidsson, 1988). The latter has been considered to be analogous to laminae I and/or II of the medullary dorsal horn (Chan-Palay, 1978; Panneton & Burton, 1981; Takemura et al. 1991). In addition, some fibres from the Para V have been shown to project directly to the NTS (Menetrey & Basbaum, 1987), which is an important relay centre for visceral afferents from the trigeminal, geniculate and superior vagal ganglia (Kerr, 1962; Beckstead & Norgren, 1979; Jacquin et al. 1983). The present study has shown a direct projection from IANs to NTS. In view of this afferent connection, it may be suggested that the Para V and NTS could be involved in the integration of afferent inputs from the auricular skin area as a part of the somatovisceral reflex.

In summary, the terminal fields of the central projections of IANs may be divided into 3 functional groups. The 1st group is concerned with thermoreception and nociception, i.e. Vc, Para V and upper cervical segments, the 2nd group of nuclei is associated with the relay of visceral sensation, i.e. Para V and NTS, and the 3rd is related to the discriminatory aspect of touch, involving Vp, Vo, Vi, Nu X and NC. Concerning the connection between the spinal trigeminal nuclei and preganglionic sympathetic neurons of the cord, it would be pertinent to note that a descending projection from the Vc and C1 to the superficial or deep dorsal horn of the entire length of the spinal cord has been reported in the cat and rat (Matsushita et al. 1981; Ruggiero et al. 1981; Tavares & Lima, 1994). Furthermore, Craig (1993) also specifically demonstrated that the superficial laminae of the cervical enlargement project directly to the thoracolumbar sympathetic nuclei in the cat and monkey. Despite these results and ours, it remains to be ascertained if there exists a neural connection between the IANs and the sympathetic nuclei in the spinal cord through the trigeminospinal descending pathway.

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