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Arrangement and number of intralaryngeal ganglia and ganglionic neurons: comparative study of five species of mammals

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Abstract

The arrangement and number of intralaryngeal ganglia and their neurons in five mammals (dog, rat, guinea pig, rabbit and cat) were examined morphologically. Intralaryngeal ganglions were situated mainly in branches of the internal branch of superior laryngeal nerve (Int-SLN), dorsal and/or dorsolateral to the posterior cricoarytenoid muscle, and around the inferior laryngeal nerve in dogs, rats, guinea pigs and cats, but they were identified at the branching out point of the Int-SLN exclusively in rabbits. The ganglion of each animal was spindle-shaped, with a surrounding fibrous capsule, and it contained many ganglionic neurons, vessels and connective tissue cells. The ganglionic neuron was oval-shaped and had a round nucleus: the diameter was smaller (20–25 μm) in the rat than in the other mammals (25–30 μm). More than 80 per cent of ganglionic neurons occurred in the supraglottis of all the animals except the rat. In the rat, this value was approximately 40 per cent.

Key words: Larynx; Ganglia; Mammals

Introduction

It has been accepted that laryngeal parasympathetic preganglionic nerve fibres arise from the dorsal motor nucleus of the vagus nerve, descending from the medulla oblongata through the vagus nerve and connecting with the postganglionic neurons which are located close to the larynx or in the laryngeal framework and supplying the vessels and glands.

The existence of the ganglia in the laryneal framework of the human and dogs was described in the early twentieth century (Elze, 1923; Sugano, 1929; Nonidez, 1931; Lemmere, 1932). In the human (newborn and premature) the location of intralaryngeal ganglia was determined by German physicians (Watzka, 1963; Kleinsasser, 1964; Schönberger, 1966; Jansen and Netter-Marbell, 1967). According to their reports, the laryngeal ganglia appeared in the internal branch of the superior laryngeal nerve (Int-SLN) in the plica ventricularis and in the vicinity of a branch of the inferior laryngeal nerve (ILN) between the cricoid cartilage and the inferior horn of the thyroid cartilage (Sugano, 1929; Watzka, 1963; Kleinsasser, 1964; Jansen and Netter-Marbell, 1967). In the dog, Sugano (1929) reported that laryngeal ganglia were observed along the Int-SLN, in Galen's anastomosis and around the ILN. In the previous literature, no remarkable reports concerning the intralaryngeal ganglia are to be found. The authors have investigated not only the arrangement of intralaryngeal ganglia and their neurons but also the functional features of ganglionic neurons in the cat by means of several methods (Yoshida *et al.*, 1992; Shimazaki, 1993; Yoshida *et al.*, 1993). However, in other animals, including dogs, information on distribution, number and size of ganglia and ganglionic neurons in the larynx is still lacking. The main purpose of the present study was to demonstrate the precise localization and to compare morphological differences of laryngeal ganglia and their neurons in the four mammals which are usually employed in neuroanatomical examinations.

Material and methods

Five of each animal, dogs (weighing 2500 to 3500 g), rats (weighing 250 to 350 g), guinea pigs (weighing 600 to 800 g) and rabbits (weighing 1800 to 2300 g) were used for the present study. All animals were anaesthetized using intramuscular ketamine (30–40 mg/kg) and xylazine hydrochloride (0.5–1.0 mg/kg) and perfused transcardially with 10 per cent buffered formaldehyde. The larynx was removed from each animal and embedded in paraffin wax. Serial horizontal sections 6 μm thick were cut and stained with haematoxylin and eosin. All sections were examined by light microscopy. Gang-

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lionic neurons in which a nucleus could be clearly distinguished were counted in each sample.

Results

The results of the present study are described together with those of our previous investigation in cats (Yoshida *et al.*, 1992; Shimazaki, 1993; Yoshida *et al.*, 1993).

The arrangement and number of intralaryngeal ganglia and ganglionic neurons in each animal

Dog. One or two large ganglia consisting of 40 to 70 ganglionic cells per ganglion were found along the branch of the Int-SLN in the rostral part of the paraglottic space and also close to the inner aspect of the lamina of the thyroid cartilage (Figure 1); one or two medium ganglia involving 25 to 40 ganglionic neurons per ganglion were found dorsal to the posterior cricoarytenoid muscle (PCA). In addition, one or two small ganglia composed of 10 to 25 ganglionic cells, were identified around the ILN and also dorsolateral to the PCA (Figure 2). The ganglionic neurons totalled 300 to 450. About 80 per cent of them appeared in the supraglottis.

Rat. One or two small ganglia having 20 to 45 ganglionic neurons in each ganglion were observed at the region where the Int-SLN branches into the anterior branch and also immediately medial to the thyroid cartilage (Figure 3); three or four small ganglia containing 15 to 30 ganglionic neurons per ganglion were found around the ILN and also dorsolateral to the PCA (Figure 4). The total number of ganglionic perikarya was 250 to 320. Forty per cent of the ganglionic cells appeared in the supraglottis.

Guinea pig. Two or three small ganglia including five to 20 ganglionic perikarya in each ganglion were found in the branch of Int-SLN and also in the vicinity of the inner aspect of the lamina of the thyroid cartilage, as well as lateral to the epiglottic petiole at the level rostral to the glottis (Figure 5); one to two small ganglia consisting of five to 10 ganglionic cells in each ganglion were seen around the ILN and also dorsolateral to the PCA (Figure 6). The ganglionic neurons were 100 to 150 in total. Approximately 80 per cent of them were found in the supraglottis.

Rabbit. Two to four small ganglia having five to 20 ganglionic cells were found at the region where the Int-SLN bifurcates in the supraglottis and also in between the arytenoid cartilage and the lamina of the thryoid cartilage (Figure 7). In other parts of the laryngeal framework, definite ganglion were not observed (Figure 8). However, some ganglionic cells appeared sparsely in the nerve bundles of the ILN or its branches without forming a ganglion. The total number of ganglionic perikarya was 100 to 150.

Cat. Three to four large ganglia containing 50 to 80 ganglionic neurons were identified in the anterior branch of Int-SLN at the rostral portion of the paraglottic space and also close to the inner aspect of

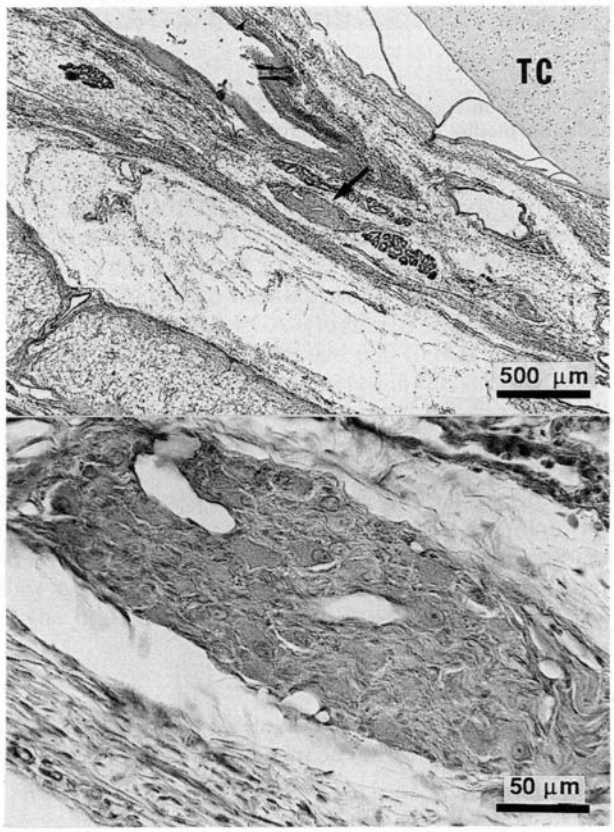

FIG. 1

Photomicrographs of a large intralaryngeal ganglion (arrowed) in the rostral part of the paraglottic space of the dog. TC = thyroid cartilage.

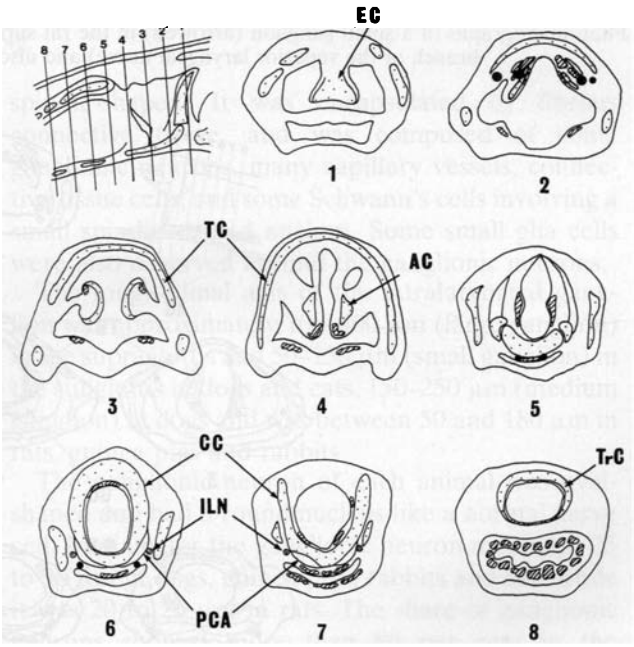

FIG. 2

Schematic drawings of the arrangement of intralaryngeal ganglia in cross-section at eight different levels of the larynx in the dog rostrocaudally. EC epiglottic cartilage; TC = thyroid cartilage; AC = aryteroid cartilage; CC = cricoid cartilage; Tr C = tracheal cartilage; ILN = interior laryngeal nerve; PCA = posterior cricoarytenoid muscle. Large black circle, large ganglion (100–400 μm); small black circle, small ganglion (50–180 μm).

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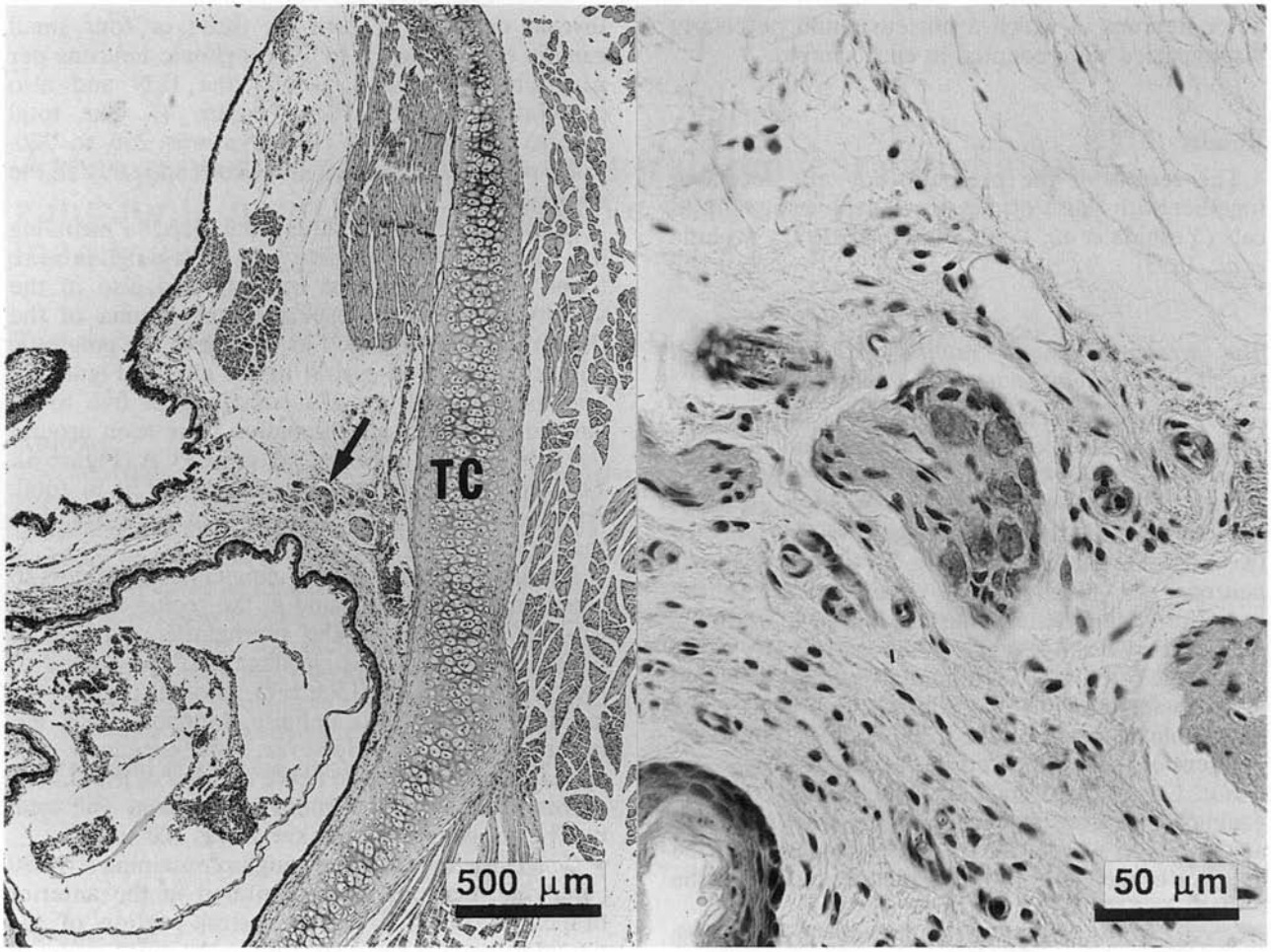

FIG. 3

Photomicrographs of a small ganglion (arrowed) in the rat supraglottis. Ganglion appeared in a branch of the Int-SLN (internal branch of the superior laryngeal nerve) and also immediately medial to the TC (thyroid cartilage).

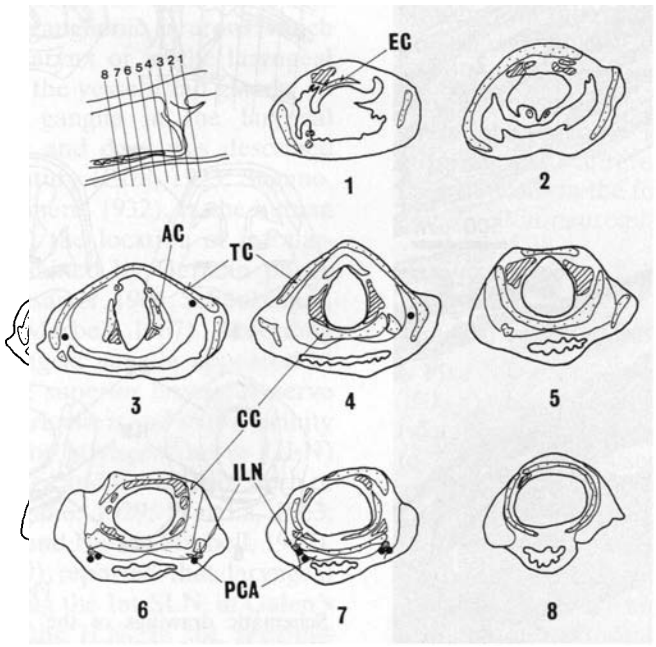

FIG. 4

Schematic drawings of the arrangement of intralaryngeal ganglia in cross-section at eight different levels of the larynx in the rat rostrocaudally. EC = epiglottic cartilage; AC = arytenoid cartilage; TC = thyroid cartilage; CC = cricoid cartilage; ILN = inferior laryngeal nerve; PCA = posterior cricoarytenoid muscle. Large black circle, large ganglion (100–400 μm); small black circle, small ganglion (50–180 μm).

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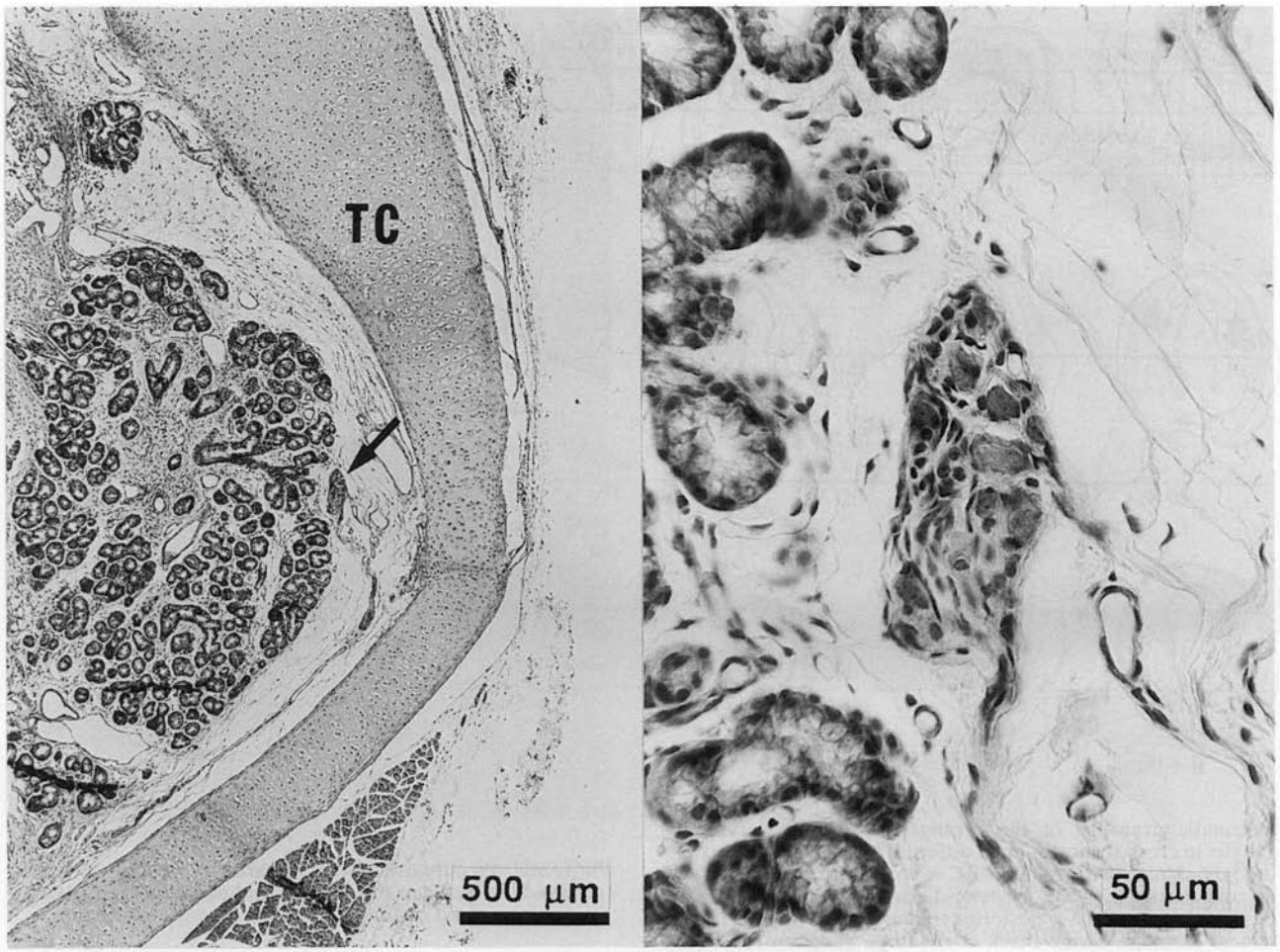

FIG. 5

Photomicrographs of a small ganglion (arrowed) rostral to the glottis and also the inner aspect of the TC (thyroid cartilage) in the guinea pig.

the thyroid cartilage. Two to three small ganglia involving five to 25 ganglionic neurons per ganglion were found dorsal to the PCA and one to two small ganglia consisting of 15 to 25 ganglionic neurons in each ganglion were found around the ILN and also dorsolateral to the PCA (Figure 9). The intralaryngeal ganglionic cells totalled 600 to 800, and more than 80 per cent of them occurred in the supraglottis.

The arrangement of intralaryngeal ganglia in dogs, rats, guinea pigs and cats was similar, but it was different in rabbits. In the former four animals, laryngeal ganglia were situated mainly in the branches of the Int-SLN, dorsal and/or dorsolateral to the PCA and around the ILN, whereas in rabbits they were recognized exclusively at the region close to the branching out point of Int-SLN.

Morphological differences between intralaryngeal ganglia and ganglionic neurons in each animal

Morphological differences such as components of the ganglion and the shape of the ganglionic cells in the laryngeal framework of five animals could not be differentiated by haematoxylin and eosin staining. The intralarygneal ganglion in each animal was localized in a nerve bundle and was round, oval or spindle-shaped. It was encapsulated by fibrous connective tissue, and was composed of some ganglionic neurons, many capillary vessels, connective tissue cells, and some Schwann's cells involving a small spindle-shaped nucleus. Some small glia cells were also observed around the ganglionic neurons.

The longitudinal axis of the intralaryngeal ganglion was approximately 100–400 μm (large ganglion) in the supraglottis and 50–150 μm (small ganglion) in the subglottis in dogs and cats, 150–250 μm (medium ganglion) in dogs and was between 50 and 180 μm in rats, guinea pigs and rabbits.

The ganglionic neuron of each animal was oval-shaped and had a round nucleus like a normal nerve cell. In diameter the ganglionic neuron measured 25 to 30 μm in dogs, guinea pigs, rabbits and cats while it was 20 to 25 μm in rats. The share of ganglionic neurons showed more than 80 per cent in the supraglottis in all animals investigated, except rat. About 60 per cent of the rat intralaryngeal ganglionic neurons occurred in the subglottic region.

The distribution, number and size of the intralaryngeal ganglia and ganglionic neurons are shown in Table I, and the arrangement of intralaryngeal ganglia is depicted schematically in cross-sections

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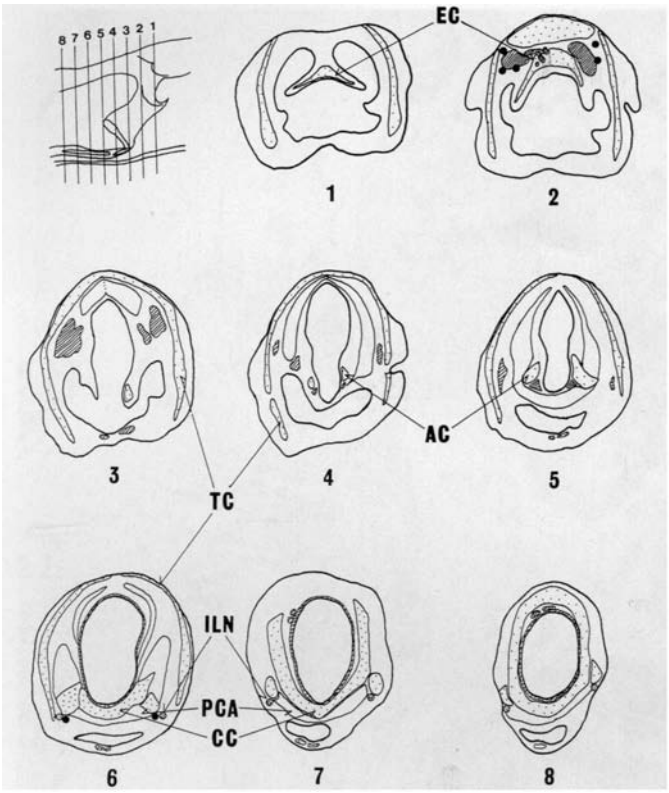

FIG. 6

Schematic drawings of the arrangement of intralaryngeal ganglia in cross-section at eight different levels of the larynx in the guinea pig rostrocaudally. EC = epiglottic cartilage; TC = thyroid cartilage; AC = arytenoid cartilage; ILN = interior laryngeal nerve; PCA = posterior cricoarytenoid muscle; CC = cricoid cartilage. Large black circle, large ganglion (100–400 μm); small black circle, small ganglion (50–180 μm).

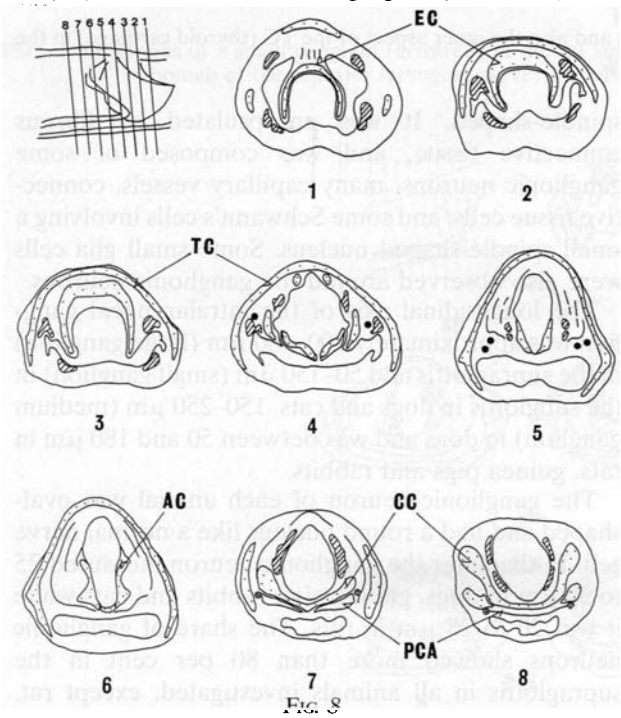

FIG. 8

Schematic drawings of the arrangmeent of intralaryngeal ganglia in cross-section at eight different levels of the larynx in the rabbit rostrocaudally. EC = epiglottic cartilage; TC = thyroid cartilage; AC - arytenoid cartilage; CC = cricoid cartilage; PCA = posterior cricoarytenoid muscle. Large black circle, large ganglion (100–400 μm); small black circle, small ganglion (50–180 μm).

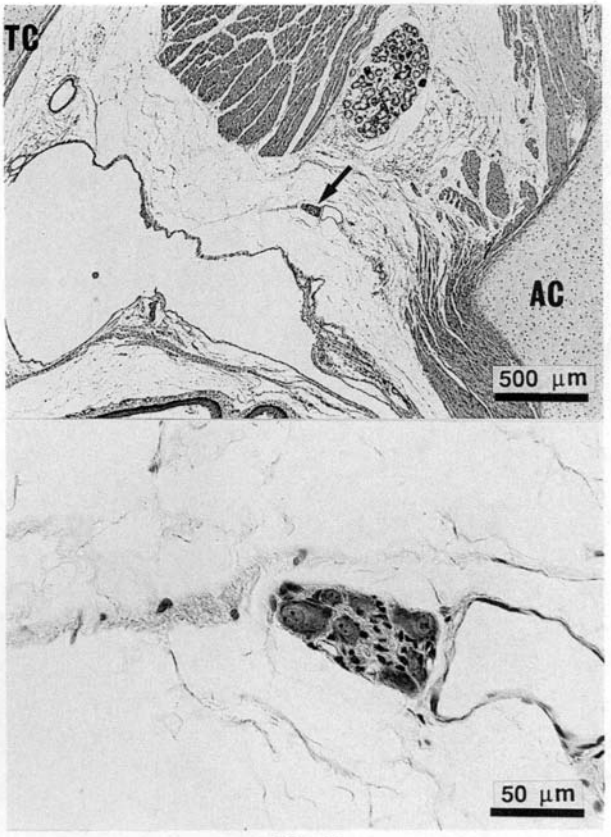

FIG. 7

Photomicrographs of a small intralaryngeal ganglion (arrowed) in between the TC (thyroid cartilage) and the AC (arytenoid cartilage) and rostral to the glottis.

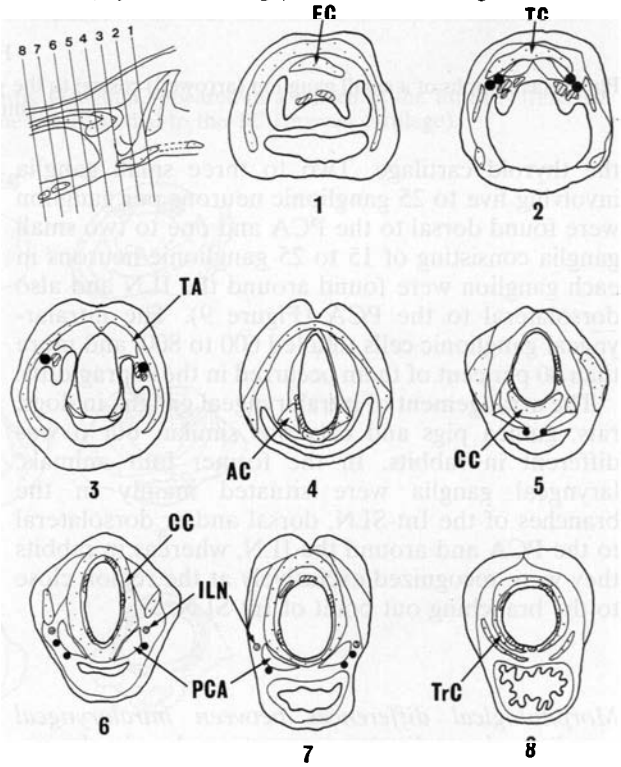

FIG. 9

Schematic drawings of the arrangement of intralaryngeal ganglia in cross-section at eight different levels of the larynx in the cat rostrocaudally. EC = epiglottic cartilage; TC = thyroid cartilage; TA = thyroarytenoid muscle; AC = arytenoid cartilage; CC = cricoid cartilage; ILN = inferior laryngeal nerve; PCA = posterior cricoarytenoid muscle; TrC = tracheal cartilage. Large black circle, large ganglion (100–400 μm); small black circle, small ganglion (150–180 μm).

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TABLE I

SUMMARY OF DISTRIBUTION, NUMBER AND SIZE OF THE INTRALARYNGEAL GANGLIA AND THEIR NEURONS IN FIVE MAMMALS

	Ganglion			Ganglionic neurons			
Animal	Location	Size (¼m)	Number (one side)	Size (¼m)	Number per ganglion	Total number	Share of ganglionic neurons
Dog	Rostral part of paraglottic space	Large (150–400)	1–2		40–70		80%
	Dorsal to PCA	Medium (150–250)	1–2	25–30	25–40	300–450	20%
	Around ILN	Small (50–150)	1–2		10–25		
Rat	Branching point of Int-SLN	Small (50–180)	1–2		20–45		40%
				20–25		250–230	
	Around ILN	Small (50–100)	3–4		15–30		60%
Guinea pig	Branch of Int-SLN	Small (50–150)	2–3		5–20		80%
				25–30		100–150	
	Around ILN	Small (50–100)	1–2		5–10		20%
Rabbit	In branching point of Int-SLN	Small (50–150)	2–4	25–30	5–20	100–150	
Cat	Paraglottic space	Large (100–400)	3–4		50–80		80%
	Dorsal to PCA	Small (50–150)	2–3	25–30	5–25	600–800	20%
	Around ILN		1–2		15–25		

of eight different levels of the larynx in the five animals in Figures 2, 4, 6, 8 and 9.

Discussion

Investigations regarding the intralaryngeal ganglia are shown in Table II. In the early 1900s, Elze (1923) reported for the first time, the existence of human intralaryngeal ganglia in the superior laryngeal nerve (SLN). He speculated that the nature of these ganglionic neurons was sensory on account of the morphological features of the cell. Then, canine intralaryngeal ganglia were identified in the Int-SLN, Galen's anastomosis and around the inferior laryngeal nerve (ILN) (Sugano, 1929; Nonidez, 1931; Lemmere, 1932). In particular Sugano (1929) detected localization of ganglia accurately. According to his descriptions, large ganglia were found in the branching point of Int-SLN, small ganglia in Galen's anastomosis and around the ILN at the level

TABLE II

A COURSE OF INVESTIGATIONS REGARDING THE INTRALARYNGEAL GANGLIA

		Results	
Investigations	Year	Location	Nature
Elze (human)	1923	SLN (ramus anastomoticus)	Sensory
Sugano (dog)	1929	Branch of Int-SLN (large) Galen's anastomosis (small) Branching point of ILN (small)	Parasympathetic
Nonidez (dog)	1931	RLN (its entrance into larynx)	Parasympathetic
Lemmere (dog)	1932	SLN, ramus anastomoticus, intrinsic laryngeal muscles	Parasympathetic
Watzka (human)	1963	Plica ventricularis	
Kleinsasser (human)	1964	Between cricoid cartilage and thyroid cartilage	Same structure as carotid body
Schönberger (newborn)	1966	Plica ventricularis	
Jansen *et al.* (premature)	1967	Plica ventricularis, between cricoid cartilage and thyroid cartilage	Parasympathetic
Afifi (human)	1971	Epiglottis (level of arytenoid cartilage)	Parasympathetic
Lawson and Zak (adult, newborn)	1974	Supraglottis, between cricoid cartilage and 1st tracheal ring	Chemoreceptor
Ramaswamy and Kulasekaran (adult, fetus)	1974	Along Int-SLN and its branches (found macroscopically)	Parasympathetic
Domeij *et al.* (rat)	1991	No description	Parasympathetic
Yoshida *et al.* (cat)	1992	Branches of Int-SLN, posterior to PCA, around ILN	No description
Tsuda *et al.* (cat)	1990	Along Int-SLN (laryngeal ventricle)	Parasympathetic
Yoshida *et al.* (cat)	1993	Inner aspect of thyroid cartilage, dorsal to PCA	Mainly parasympathetic
Shimazaki (cat)	1993	Rostral part of paraglottic space, dorsal to PCA, around ILN	Partly sympathetic and sensory

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of the bifurcation of the anterior and posterior branches (Sugano, 1929). On the other hand, Sugano (1929) and Lemmere (1932) pointed out the presence of these ganglia in the intrinsic laryngeal muscles (the cricoarytenoid muscle and the PCA) except for their existence in the laryngeal nerves. In the dog, functional features of laryngeal ganglionic neurons were considered as parasympathetic morphologically (Sugano, 1929; Nonidez, 1931; Lemmere, 1932).

In the human, Watzka (1963) found ganglia (0.1-0.3 mm in diameter) in the vicinity of Int-SLN in the plica ventricularis. The following year, Kleinsasser (1964) revealed ganglion (0.3–0.4 mm in diameter) close to the ILN in between the inferior horn of the thyroid cartilage and the cricoid cartilage. Then, Schönberger and other authors verified the existence of intralaryngeal ganglia in newborn and premature babies (Schönberger, 1966; Jansen and Netter-Marbell, 1967; Lawson and Zak, 1974). In 1971, a very small ganglion (about 1 × 0.5 mm) embedded between nerve fibres of the branches of Int-SLN (at the level corresponding to the apex of the arytenoid cartilage) that inervate the aryepiglottic fold and the lower part of the epiglottis was recognized, and named the epiglottic ganglion by Afifi (1971). This ganglion was situated on both sides of the epiglottis. A relatively large ganglion (1–5.5 × 1–4 mm) was disclosed constantly in the branches of the Int-SLN at a location approximately 1.0 cm from the thyroid notch by the investigation of Ramaswamy and Kulasekaran, (1974). In the macroscopic observation of more than 100 human larynges, they defined the presence of a single ganglion on each side in the majority of specimens, but two or more ganglia in a few cases. They also stated that no ganglia were observed in the other mammals such as dogs, cats, rabbits, goats and monkeys macroscopically (Ramaswamy and Kulasekaran, 1974). In the human, some conflicting descriptions concerning the size of ganglia in the supraglottis were found. The functional features of these human intralaryngeal ganglia have been discussed with regard to location and shape and size of ganglionic cells as sensory, particularly chemoreceptor and parasympathetic (Elze, 1923; Watzka, 1963; Kleinsasser, 1964; Jansen and Netter-Marbell, 1967; Afifi, 1971; Lawson and Zak, 1974; Ramaswamy and Kulasekaran, 1974).

In the laryngeal ganglionic cells of the rat Domeij *et al.* (1991) studied immunohistochemically that enkephalin was coexhibited with neuropeptide Y and vasoactive intestinal polypeptide. These findings were considered parasympathetic in nature. However, a morphological description of the arrangement and size of the ganglia was absent.

As regards the feline laryngeal ganglia, we have investigated not only arrangement, shape, size and number of ganglia and their neurons, but also the nature of ganglionic neurons (Shimazaki, 1993; Tanaka *et al.*, 1993; Yoshida *et al.*, 1993). Tsuda *et al.* (1990) also reported that at least three or four local ganglia (about 0.5 mm in diameter) were located mainly along the Int-SLN and around the laryngeal ventricle, and the ganglionic neuron was about 30 μm in diameter and had an elliptical shape. Their results for supraglottic ganglia is in agreement with ours. The functional significance of ganglia in the feline laryngeal framework was detected as parasympathetic chiefly with reference to immunoreactivity.

In the guinea pig and rabbit, no studies of ganglia and their neurons in the larynx can be found in previous literature.

In the present study, localization of laryngeal ganglia of the supraglottis was similar in all animals investigated while that of the subglottis was different. Especially, in the rabbit, no laryngeal ganglia in the subglottis were seen. These ganglia in each animal were localized in nerve bundles and were round, oval- or spindle-shaped. Each ganglion was encapsulated by fibrous connective tissue and involved some ganglionic neurons and Schwann's cells, many connective tissue cells, small glia cells and many capillary vessels. However, we were not able to observe the existence of paraganglia by haematoxylin and eosin staining. The ganglionic neurons were aggregated in the ganglion of the dog and cat while they were sparse in the other animals. The size of the ganglion was larger in the dog and cat than in the other animals, and also larger in the supraglottis than in the subglottis in each animal.

Regarding the relationship between the distribution of the laryngeal glands (see Figures 2, 4, 6, 8 and 9) and the intralaryngeal ganglia, laryngeal ganglia of the dog, guinea pig and cat were located in the vicinity of the glands whereas those of the rat and rabbit were situated far from the glands. In general, the ganglia appeared close to the glands. The authors could not speculate on the cause of the findings in the rat and rabbit.

In each animal, the ganglionic neuron was oval-shaped and had a round nucleus like a normal nerve cell. The size of the neuron was smaller (20 to 25 μm) in the rat than in the other mammals. The ganglionic neuron in the other animals was 25 to 30 μm in diameter. Total number of laryngeal ganglionic neurons was 600 to 800 in cats, 300 to 450 in dogs and 250 to 320 in rats. In the guinea pig and rabbit, ganglionic neurons totalled 100 to 150 and were only one-fifth of the cat's intralaryngeal neurons. More than 80 per cent of ganglionic neurons occurred in the supraglottis of all animals, except the rat. A difference in total number and share of ganglionic neurons in the animals examined is indistinct, probably because of the difference in species.

Finally, in our previous investigations of the cat (Yoshida *et al.*, 1992; Shimazaki, 1993; Yoshida *et al.*, 1993) the arrangement of intralaryngeal ganglia and their neurons and functional aspects of ganglionic perikarya have indicated that the ganglionic neurons are mainly parasympathetic and also partly sympathetic and sensory. In other mammals, to determine whether similar characteristics are shared, further study is needed. The present study is one of a series

of investigations concerning the role of intralaryngeal ganglionic neurons in mammals.

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