The Journal of Laryngology & Otology
April 2001, Vol. **115,** pp. 257–266

Review Article

Comparative study of the laryngeal innervation in humans and animals employed in laryngeal transplantation research

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Abstract
Laryngeal transplantation is receiving increased attention. Re-innervation of the transplanted larynx is critical for a successful functional outcome. Different anatomical models (dog, cat, rat, pig) have been employed for experimental purposes. Interspecies similarities and differences are important for extrapolating the experimental results to humans. We present a review of the anatomical course and regional branching patterns of the laryngeal nerves in both humans and animals currently being employed in laryngeal transplantation. The clinical and surgical implications are also discussed.

Key words: Larynx; Nerve fibres; Transplantation; Animals, Laboratory

Introduction

Organ transplantation has made significant progress in recent years. Refinements in surgical techniques, newly-developed immunosuppressive drugs and regimens,[1] selective re-innervation, extended organ preservation and improved peri-operative management have brought new possibilities for transplantation. Increasingly, transplantation is being viewed as a means of increasing both the quality of life as well as the quantity.[2] The loss of a functioning larynx, although not so essential for life, causes devastating lifestyle changes resulting in permanent cosmetic and psychosocial disability.

The notion of returning phonation and respiratory function by transplanting larynges has fascinated and challenged laryngologists since the 1950s. On January 4, 1998, Strome performed the first true human laryngeal transplantation and two years on, the patient continues to do well.[3] However, before laryngeal transplantation can become a practical reality it is essential to answer both technical and immunological questions. Re-inneveration of the transplanted larynx is of special importance for a successful outcome. A major step towards achieving this goal is the establishment of a proper animal model. Dogs,[4] cats[5] and rats[6] have been widely used as preclinical models and a thorough knowledge of their laryngeal anatomy, particularly innervation, is a pre-requisite for studying interventions in the larynx. Although the vast majority of experimental work on laryngeal transplantation has involved these animals, the introduction of the pig model may also provide an attractive alternative for future experimental work.[7]

We present a review of the anatomical course and regional branching patterns of the laryngeal nerves in both humans and animals currently being employed in laryngeal transplantation research (dog, cat, rat, pig). An attempt is made, not only to delineate the laryngeal innervation and its clinical

TABLE I
ABBREVIATIONS

SLN	Superior laryngeal nerve
RLN	Recurrent laryngeal nerve
InSLN	Internal branch of the superior laryngeal nerve
ExSLN	External branch of the superior laryngeal nerve
AnRLN	Anterior branch of the recurrent laryngeal nerve
PoRLN	Posterior branch of the recurrent laryngeal nerve
IA branch	Interarytenoid branch
CrLN	Cranial laryngeal nerve
InCrLN	Internal branch of the cranial laryngeal nerve
ExCrLN	External branch of the cranial laryngeal nerve
CaLN	Caudal laryngeal nerve
PaRLN	Para-recurrent laryngeal nerve
DAB-CrLN	Dorsal accessory branch of the cranial laryngeal nerve
NG	Nodose ganglion
CT muscle	Cricothyroid muscle
IPC muscle	Inferior pharyngeal constrictor muscle
PCA muscle	Posterior cricoarytenoid muscle
LA muscle	Interarytenoid muscle
TA muscle	Thyroarytenoid muscle
LCA muscle	Lateral cricoarytenoid muscle

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Accepted for publication: 19 October 2000.

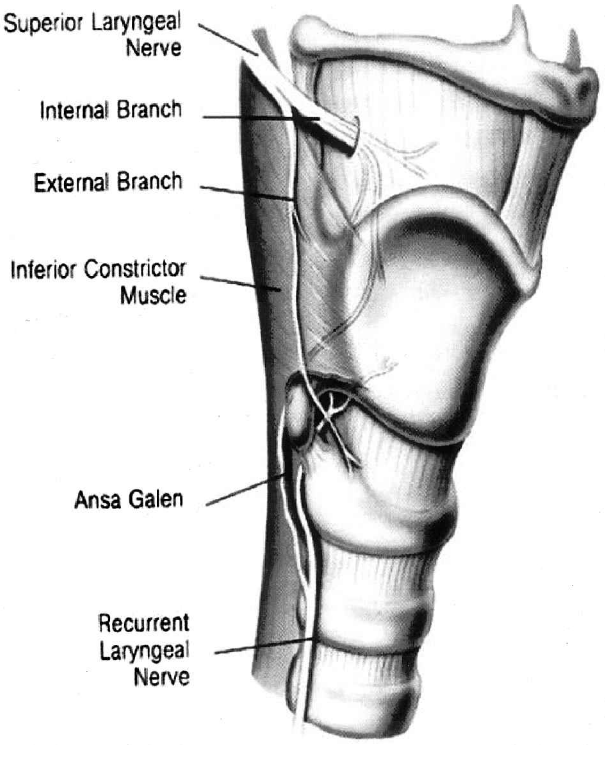

FIG. 1
Schematic drawing of the human laryngeal innervation.

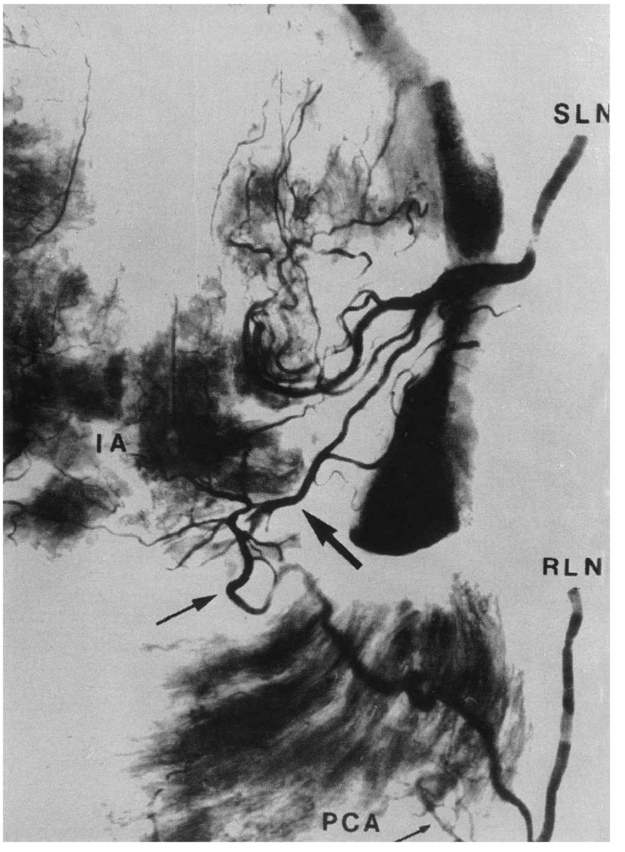

FIG. 2
Posterior view of the larynx demonstrating the right recurrent laryngeal nerve (RLN), the nerve branches to the posterior cricoarytenoid muscle (PCA) (small arrow) and the interarytenoid muscle (IA) (medium arrow) and the anastomosis between the RLN and the superior laryngeal nerve (SLN) (large arrow) (with permission from Figure 1, Sanders I, Li Y, Biller H. Axons enter the human posterior cricoarytenoid muscle from the superior direction. *Arch Otolaryngol Head Neck Surg* 1995; **121**: 755; Copyright 1995, American Medical Association).[18]

significance, but also to clarify possible interspecies similarities and differences. All abbreviations used in the text are explained in Table I.

The human larynx

The human larynx receives innervation from the vagus nerve via the superior (SLN) and recurrent (RLN) laryngeal nerve (Figure 1).

The SLN issues from the caudal end of the nodose ganglion and travels inferiorly, initially under and then medial to the internal carotid artery. The nerve divides into a large internal (InSLN) and a small external branch (ExSLN), approximately 1.5 cm below the nodose ganglion[8] (NG) or further caudally either at the level of the greater cornu of the hyoid bone or within the carotid bifurcation.[9] Rarely, the SLN may branch immediately before the internal branch pierces the thyrohyoid membrane,[10] while occasionally the two branches may originate from the ganglion itself.[8]

The InSLN (1–2 mm in diameter) descends above the superior laryngeal artery on the thyrohyoid membrane, that it pierces through a hiatus at the upper edge of the inferior pharyngeal constrictor (IPC) muscle. Within the thyrohyoid membrane or even external to it, it divides into several branches (two to five), descending along the aryepiglottic fold.[9] The most superior branches supply the mucosa of the vallecula and the epiglottis (sensory fibres). Occasionally, an anastomosis occurs over the superficial surface of the thyroarytenoid (TA) muscle resulting from the connection of a descending branch from a superior branch of the InSLN and an ascending branch from the anterior branch of the RLN (thyroarytenoid anastomosis).[11] The middle divisions descend in the medial wall of the pyriform sinus, supply the intralaryngeal mucosa (posterior commissure and subglottis) with sensory fibres and the interarytenoid muscle with motor fibres anastomosing the arytenoid branches of the RLN (Figure 2). This anastomosis usually occurs within the muscle[12] or occasionally superficially on its posterior surface.[13] Rarely, a superior branch arising from the deep part of this anastomosis communicates with an inferior branch coming from each RLN, just before they enter the larynx, forming a cricoid anastomosis in front of the cricoid lamina.[11] This cricoid anastomosis probably supplies the mucosa of the posterior surface of the subglottis. The most inferior branch of the InSLN runs postero-inferiorly, on the surface of the posterior cricoarytenoid (PCA) muscle and joins the posterior branch of the RLN (PoRLN) forming Galen's anastomosis. Galen's anastomosis exhibits several patterns – single trunk, double trunk or plexus.[4] Its fibres are proprioceptive, sensory

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supplying the mucosa of the postcricoid area and the cervical oesophagus.[15] Rarely, the inferior division may run directly to the pharyngeal and oesophageal mucosa without joining the RLN.

The ExSLN (0.2 mm in diameter) passes posterior to the sternothyroid muscle deeper to the superior thyroid artery. It descends on the fascia (85–90 per cent) or within the inferior pharyngeal constrictor (IPC) muscle (10–15 per cent) sending branches to the pharyngeal plexus.[16] Occasionally, close to the superior third of the thyroid lamina the ExSLN communicates with the main trunk of the InSLN through an anastomosis running throughout a thyroid foramen.[11] At the level of the oblique line of the thyroid cartilage or just under its lower edge (depending on the size of the thyroid gland), it curves anteriomedially and reaches the cricothyroid (CT) muscle, lying medial to the superior thyroid artery.[8] However, occasionally the nerve may be adherent to the artery and its branches (15 per cent), looped around them (six per cent) or even more rarely around the branches of the superior thyroid vein.[16] Before entering the CT muscle the nerve bifurcates supplying both the oblique and the rectus bellies of the muscle (motor fibres). This nerve-branching pattern is characterized by frequent anastomoses. Although the main branches pass through the centre of the bellies, secondary extensions travel to the superior and inferior extent.[17] From the lower third of the ExSLN small motor branches supply the lower part of the IPC muscle and communicate with the superior cardiac nerve and the superior cervical ganglion.

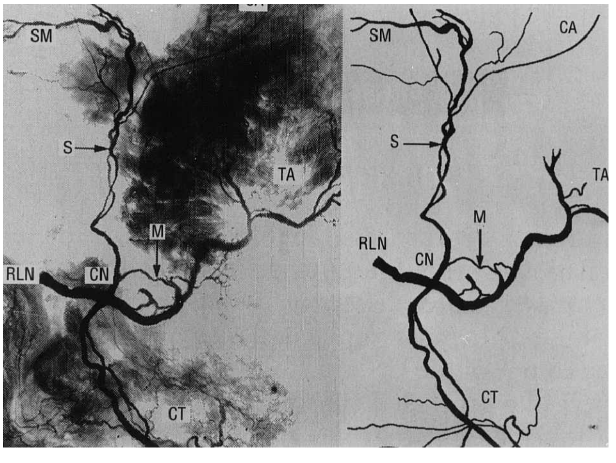

FIG. 3

Left: Neural connection (CN) between the superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN). An extension of the external superior laryngeal nerve (ES) passes through the cricothyroid muscle (CT) to emerge as the CN. The CN bifurcates into two branches: the sensory branch (arrow with S) passes through the thyroarytenoid muscle (TA) to terminate in the subglottic mucosa (SM) and cricoarytenoid joint (CA) while the motor branch (arrow with M) joins the recurrent laryngeal nerve (RLN) and terminates within the TA muscle. Right: Drawing of Figure 3, left (with permission from Figure 5, Wu BL, Sanders I, Mu L, Biller F. The human communicating nerve. An extension of the external superior laryngeal nerve that innervates the vocal cord. *Arch Otolaryngol Head Neck Surg* 1994; **120**: 1324, Copyright 1994, American Medical Association).[43]

An interconnection between an extension of the ExSLN and the anterior branch (AnRLN) of the RLN has also been described within the TA muscle.[18] Sañudo *et al.* named this communication cricothyroid anastomosis because it passes through the cricothyroid muscle and space.[11] The function of this interconnection is not clearly defined although it seems to have motor fibres for the TA muscle and sensory fibres for the subglottic mucosa of the vocal folds (Figure 3).

The RLN (1 mm in diameter) derives from the vagus nerve. On the right side it winds around the subclavian artery and gradually runs closer to the trachea as it ascends towards the tracheo-oesophageal groove. On the left side, it passes lateral to the ligamentum arteriorsum behind the aortic arch and enters the tracheo-oesophageal groove in the chest running parallel to the trachea in a slightly deeper plane than on the right. During their ascent towards the larynx, branches (with motor and/or sensory fibres) are distributed to the oesophagus, the trachea and the IPC muscle, more numerous on the left. Rarely, the nerve may pass directly to the larynx (inferior laryngeal nerve) without recurring.[19] At the lower pole of the thyroid gland, the nerve may cross either posteriorly or anteriorly to the inferior thyroid artery or may pass between its branches. On the right, there is an equal chance of finding the nerve in each of these locations, while on the left it is more likely to lie posteriorly to the artery.[20]

Most older textbooks described the RLN entering the larynx as a single trunk through, or under, the IPC muscle, closely behind the cricothyroid joint.[21] However, in the majority of cases the nerve divides extralaryngeally giving an anterior motor branch (AnRLN) and a posterior, more medial sensory one (PoRLN). These two branches usually have a similar diameter, if not, the anterior branch is thicker.[13] Rarely, a third thin branch arising from the PoRLN is present passing under the IPC muscle and disappearing rapidly into the hypopharyngeal mucosa[22] or supplying the IPC muscle of the pharynx without entering the hypopharynx.[15] The level of this division is variable, commonly occurring just below the IPC muscle, close to the inferior thyroid cornu, or up to 20 m below.[22] Occasionally, it can be 4 cm below the muscle,[23] at the junction of the middle and lower thirds of the thyroid gland[24] or even at the lower pole of the gland.[25]

The AnRLN approaches the larynx behind, and close to, the posterior face of the cricothyroid joint, separated from its capsule by only 1–2 mm of connective tissue. Occasionally, it may enter the larynx anterior to it. At the level of the inferior thyroid cornu, the nerve gives off at least two main branches, passing medially between the PCA muscle and the posterior lamina of the cricoid cartilage. The first one supplies the PCA muscle, while the other, at the superior margin of this muscle, dips into the

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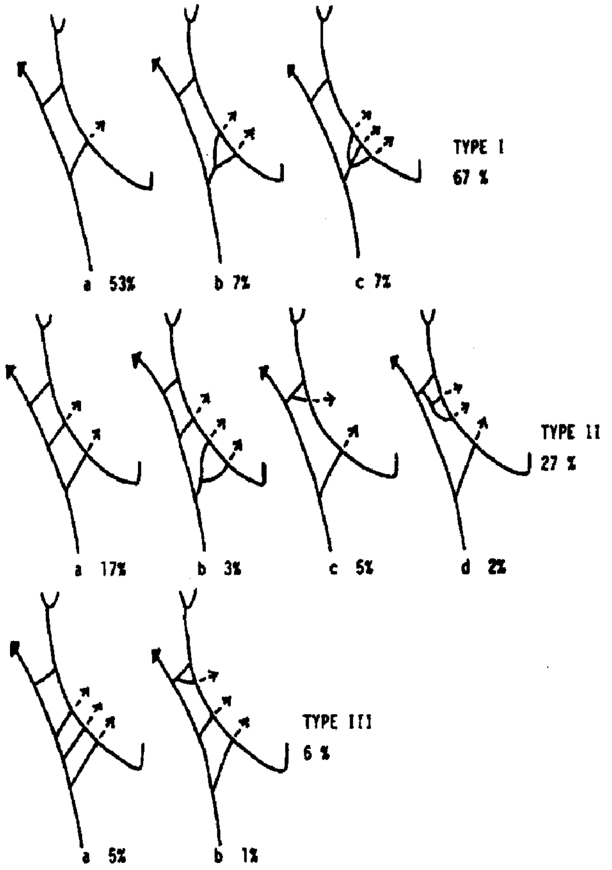

FIG. 4

The different innervation of the posterior cricoarytenoid muscle (with permission from Figure 1, Nguyen M, Junien-Lavillauroy C. An intralaryngeal anatomical study of the anterior branch of the recurrent laryngeal nerve. *Rev Laryngol* 1990; **111**: 154).[53]

fibres of the interarytenoid (IA) muscle. Therefore, the AnRLN carries both adductor and abductor fibres.

The PCA muscle is innervated either by a single nerve branch (type Ia), which bifurcates as soon as it enters the muscle, or quite frequently by two (type IIa) or even three compartmental branches (type IIIa) that arise separately off the main trunk of the AnRLN.[15] The inferior branch(es) innervate(s) the oblique belly, while the superior one supplies the horizontal belly and anastomoses with the IA branch.[26] The detailed arrangement of these nerve branches is illustrated in Figure 4.

The IA branch is almost exclusively a single one running underneath the PCA muscle before entering at the IA inferior lateral border. In the muscle it subdivides into posterior and anterior branches, which form a variable anastomotic plexus. The anterior branches further anastomoses with the ipsilateral InSLN, as already described. The IA muscle is innervated by both RLNs, which anastomose with each other across the midline with considerable overlapping.

After supplying the branches to the PCA and IA muscles, the AnRLN curves anteriorly and enters the pyriform sinus submucosally, between the thyroid lamina and the lateral laryngeal musculature, passing 2–3 cm below the muscular process of the arytenoid cartilage. It divides finally into its terminal branches for the lateral cricoarytenoid (LCA) and TA muscles. The LCA muscle is most frequently innervated by a single nerve branch forming a dense anastomotic plexus in its centre.[17] Occasionally, two or three separate AnRLN branches may supply the LCA, while a branch from the IA nerve has also been described.[27] The AnRLN terminates in the TA muscle forming the densest anastomotic network seen in any of the laryngeal muscles, especially near the vocal ligament reflecting the rapidity of the glottic closure reflex (lateral region of the TA muscle) and the delicate adjustments during phonation (medial region of the TA muscle).[17] Nguyen *et al.,* described four types of this termination: paintbrush configuration (the most frequent) single entry point termination, comb-like and star shaped.[15]

The PoRLN passes into the lateral wall of the pyriform sinus or more medially into the postcricoid area and terminates in the hypopharyngeal mucosa. During this course, it supplies many sensory branches to the laryngeal mucosa below the level of the vocal folds. Some of them anastomose with the inferior division of the InSLN, forming Galen's anastomosis.[28]

The canine larynx

The canine larynx is grossly similar to the human larynx sharing the same basic functions of phonation, respiration and glottic protection.

The cranial laryngeal nerve (CrLN) arises from the vagus nerve at the NG. It travels ventrally, medial to the common carotid artery and divides into internal (InCrLN) and external (ExCrLN) branches near the greater cornu of the hyoid bone.

The InCrLN divides into three large branches, either before or after entering the larynx through the cranial thyroid notch.[29] The first branch (anterior) supplies the superior third of the epiglottic mucosa, while the second branch (middle) innervates the remaining two thirds (mainly the laryngeal surface). The third branch (posterior) divides into two smaller branches. One of them innervates the posterior aspect of the larynx down to the level of the vocal folds, while the other joins an ascending branch from the RLN, (∀ 1 cm below the larynx), to form Galen's anastomosis. Galen's anastomosis contains sensory fibres from the trachea and the oesophagus, and possibly motor fibres to the oesophagus forming a discrete posterior branch.[30] It also carries the inhibitory input from the aortic baroreceptors explaining the arrhythmias seen during intubations, cuff inflation and other laryngeal manoeuvres.

The ExCrLN runs caudally on the lateral aspect of the pharynx and supplies with motor fibres the CT and the TA muscles.[31] It terminates with sensory fibres in the anterior subglottic mucosa.[32]

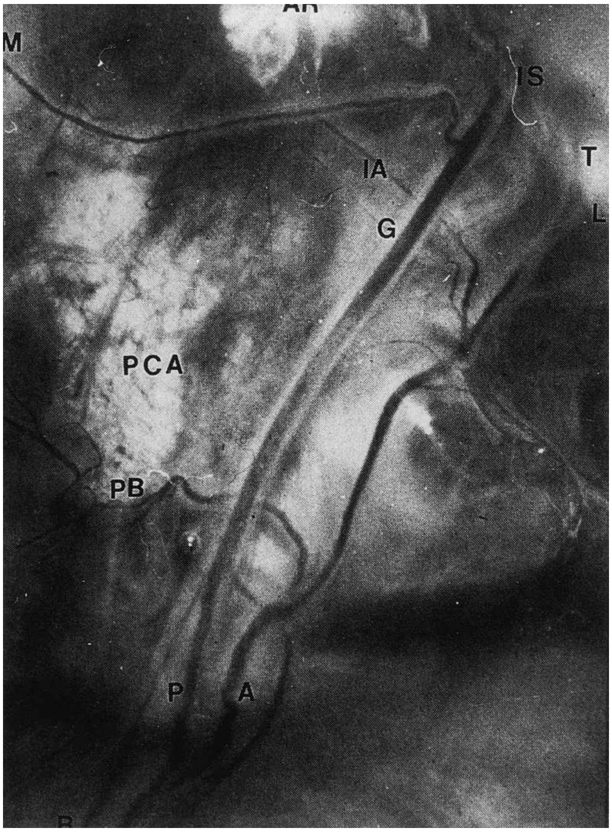

Fig. 5

Posterior view of the canine larynx demonstrating the division of the right recurrent laryngeal nerve (R) into anterior branch (A) and posterior branch (P). IA: interarytenoid branch, G: Galen's anastomosis, Is: internal branch of the superior laryngeal nerve, T: thyroarytenoid branch, L: lateral cricoarytenoid branch, PB: posterior cricoarytenoid branch; PCA: posterior cricoarytenoid muscle, AR: arytenoid cartilage (with permission from Figure 6, Wu BL, Sanders I. A technique for demonstrating the nerve supply of whole larynges. *Arch Otolaryngol Head Neck Surg* 1992; **118**: 825, Copyright 1992, American Medical Association).[27]

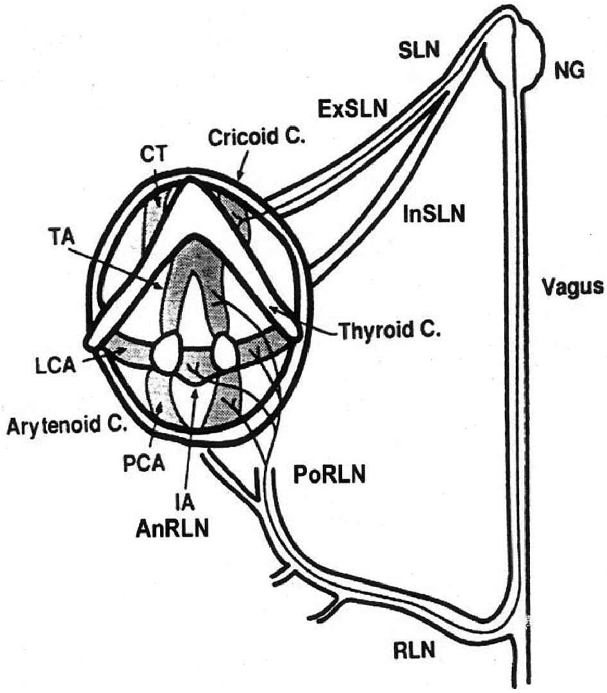

Fig. 6

Schematic drawing demonstrating the innervation of the cat larynx. NG: nodose ganglion, SLN: superior laryngeal nerve, ExSLN: external branch of the SLN, InSLN: internal branch of the SLN, RLN: recurrent laryngeal nerve, PoRLN: posterior branch of the RLN, AnRLN: anterior branch of the RLN, CT: cricothyroid muscle, TA: thyroarytenoid muscle, LCA: lateral cricoarytenoid muscle, PCA: posterior cricoarytenoid muscle, IA: interarytenoid muscle.

The RLN contains mainly motor fibres for the intrinsic laryngeal muscles (except the CT muscle) and sensory fibres for the subglottic mucosa (Figure 5). The RLN divides soon after its origin into two main branches, the caudal laryngeal nerve (CaLN), which innervates the laryngeal muscles and the pararecurrent laryngeal nerve (PaRLN), which supplies with sensory fibres the subglottis, the trachea and the oesophagus and eventually joins the CrLN.

The CaLN divides below the lower border of the PCA muscle into three smaller branches. The first ascends on the PCA muscle and joins with the posterior fibres of the InCrLN forming Galen's anastomosis. The second innervates the PCA muscle entering between its vertical and oblique bellies. Along its course to the horizontal belly, it provides multiple terminal branches forming highly complex anastomotic networks.[33] The third branch near the top of the lateral border of the PCA muscle bifurcates into medial and lateral divisions. The medial one passes between the PCA muscle and the cricoid lamina and terminates in the arytenoid and ventricular muscles. The lateral division terminates in the TA muscle, while the branch to the LCA muscle may arise from either one of the divisions of the third branch.[34] The vertical and the horizontal bellies of the PCA muscle may also receive some innervation from the third branch or the RLN, respectively. Occasionally, the branch innervating the PCA muscle may emerge in the superior aspect of the muscle and join Galen's anastomosis.[34]

The cat larynx

The course and distribution of the laryngeal nerves in the cat is basically similar to that in man (Figure 6). The CrLN issues from the rostral part of the NG, travels inferiorly (∀ 20 mm) and divides into an internal sensory (InCrLN) and an external mixed branch (ExCrLN) near the greater cornu of the hyoid bone. The InCrLN enters the larynx through the thyroid fissure and divides into anterior, middle and posterior branches.[35] The anterior branch innervates the laryngeal aspect of the epiglottis and a small anterior part of the aryepiglottic fold. The middle branch divides into two smaller branches. The first one supplies most of the aryepiglottic fold, the rostral aspect of the vocal folds, the posterolateral aspects of the arytenoid eminence and the laryngeal vestibule. The second penetrates the cricoid foramen (located in the middle of the cricoid lamina) and joins the posterior branches of the

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InCrLN and the RLN (the PaRLN) to innervate the posterior wall of the glottis and the medial aspect of the arytenoid cartilage bilaterally with ipsilateral predominance. The posterior branch divides into four smaller branches. The first one, as already mentioned, penetrates the cricoid foramen together with the PaRLN and the middle branch of the InCrLN. The second branch innervates the mucosa covering the PCA muscle in the hypopharynx, forming a dense sensory nerve plexus. The third branch runs between the TA and the LCA muscles, together with the posterior branch of the RLN, innervating the caudal aspect of the vocal folds and the subglottis. The fourth branch descends to join a branch from the PoRLN, in order to form Galen's anastomosis, providing a laryngeal pathway for aortic baroreceptor fibres.[36]

The ExCrLN descends on the surface of the IPC muscle and enters the CT muscle (motor fibres). The terminal branches pierce the CT muscle and innervate with sensory fibres the crico-tracheal interspace and the infraglottic mucosa.

The RLN divides into anterior (AnRLN) and posterior (PoRLN) branches near the caudal end of the IPC muscle. The AnRLN innervates the homolateral PCA, LCA and TA muscles (resembling the canine innervation), while the IA muscle, although poorly developed in the cat,[37] is doubly innervated by bilateral RLNs.[29] The PoRLN travels rostrally in the anterior wall of the hypopharynx and divides into two branches. The former participates in Galen's anastomosis. The latter passes between the LCA and TA muscles and joins with the posterior division of the InCrLN forming the PaRLN, which supplies sensory fibres to the posterior glottis, the caudal aspect of the vocal folds and the subglottis.[35]

The rat larynx

The innervation of the rat larynx is derived from the vagus nerve, via the CrLN and RLN (Figure 7). The CrLN arises from the NG and divides extralaryngeally into three main branches: an internal sensory (InCrLN) branch, an external mixed (ExCrLN) branch and a branch running more posteriorly called the dorsal accessory branch of the CrLN (DAB–CrLN). The InCrLN penetrates the thyroid cartilage and innervates the supraglottis. The ExCrLN supplies with motor fibres the CT muscle and occasionally the TA and the LCA muscles and terminates with sensory fibres in the anterior subglottis.[38] The DAB-CrLN innervates the subglottis area (sensory fibres) and part of the more rostral thyropharyngeus muscle, including the semicircular muscle (homologous to the human cricopharyngeus muscle), the pharyngoesophageal junction and the upper oesophagus (sensory and motor fibres). It also gives off a communicating branch to the RLN to form the rat's Galen's anastomosis, which contains both motor and sensory fibres for the oesophagus and afferent fibres from the aortic arch baroreceptors.[38] It is possible that some efferent fibres may also reach the thoracic vagus and the heart through this communicating twig joining the DAB-CrLN and RLN.[39]

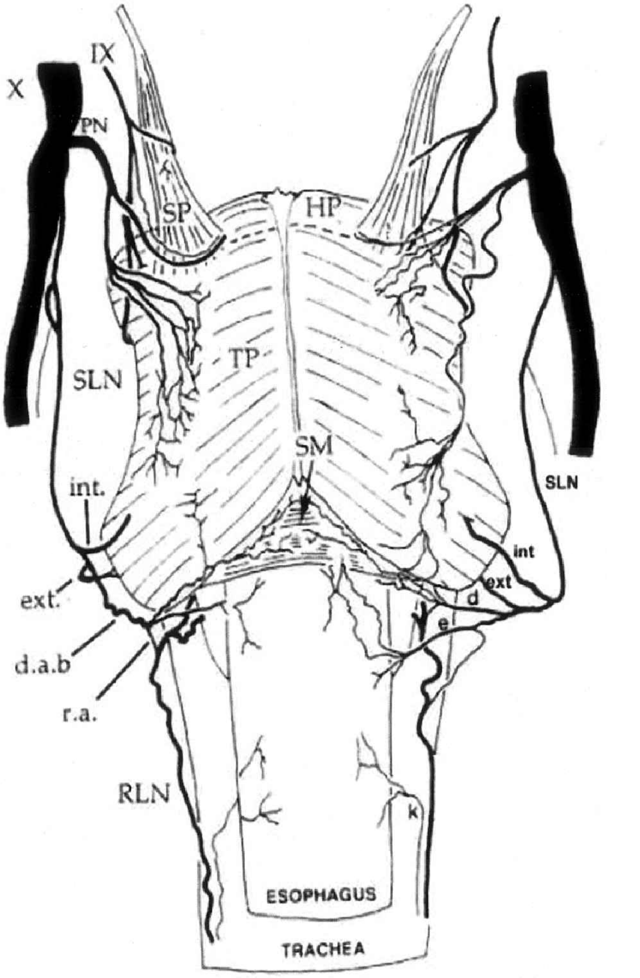

FIG. 7

Posterior view of the rat larynx, pharynx, demonstrating the typical variations in the branching and distribution of the vagus nerve (X), the pharyngeal nerve (PN), glossopharyngeal nerve (IX), superior laryngeal nerve (SLN), recurrent laryngeal nerve (RLN), internal branch of SLN (int), external branch of SLN (ext), dorsal accessory branch of SLN (d.a.b), Galen's anastomosis (r.a), branch to the semicircular muscle (d), branch from DAB-SLN to oesophagus (e), branch from RLN to oesophagus (k). SM: semicircular muscle, TP: thyropharyngeus muscle, SP: stylopharyngeus muscle (with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Figure 1, Kobler JB, Datta S, Goyal RK, Benecchi EJ. Innervation of the larynx, pharynx and upper esophageal sphincter of the rat. *J Comp Neuro* 1994; **349**: 132, Copyright 1994, Wiley-Liss Inc.)[39]

The RLN innervates the intrinsic laryngeal muscles (PCA, LCA, TA), including the two distinct muscles described in rats: the rostral cricoarytenoid and the cricovocal muscles. It also shares innervation of the cervical oesophagus with the CrLN and occasionally provides some minor innervation to the semicircular muscle and the CT muscle. A sensory component has also been demonstrated to terminate in the subglottic area.

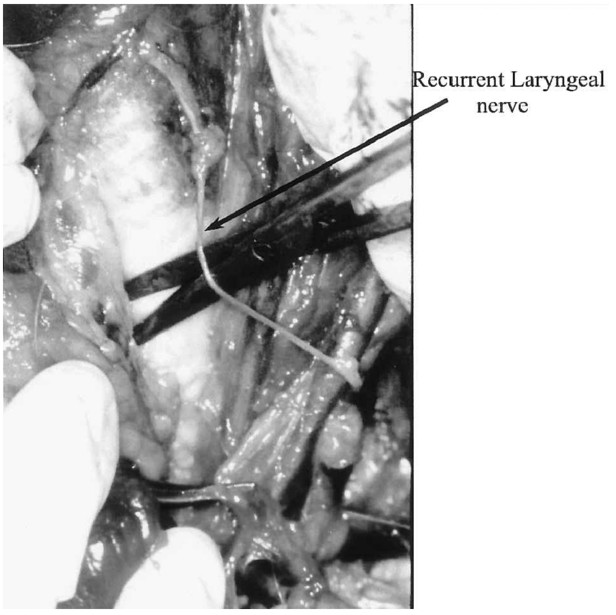

FIG. 8
Anterior view of the pig larynx demonstrating the recurrent laryngeal nerve.

The pig larynx

The nerves of the domestic pig grossly resemble those of the man in origin, branching and general distribution.

The CrLN arises from the vagus nerve at the NG and divides into internal (InCrLN) and external branches (ExCrLN). The InCrLN is the actual continuation of the CrLN and enters the larynx through the dorsal part of the thyrohyoid membrane to supply sensation in the supraglottic area. The ExCrLN is smaller and terminates in the CT muscle.

The RLN issues from the vagus nerve. The RLN enters the larynx as the caudal laryngeal nerve (CaLN), through the dorsal part of the cricothyroid membrane, between the lateral aspect of the cricoid arch and the lamina of the thyroid cartilage. It is the motor nerve to all the intrinsic muscles of the larynx with the exception of the CT muscle. It also supplies with sensory fibres the laryngeal mucosa caudal to the vocal folds (Figure. 8).

Discussion

The ability to re-innvervate the larynx with consistency is essential in the development of laryngeal transplantation. The innervation pattern of each laryngeal muscle may be the most important factor for the co-ordinate, purposeful motion required for normal laryngeal function.

However, controversy still exists in the literature concerning the precise anatomy and role of the laryngeal nerves, especially the RLN. Many classical anatomy textbooks still consider the PoRLN containing abductor, and the AnRLN adductor, motor fibres. According to them, re-anastomosis of the AnRLN with a recipient nerve (i.e. ansa cervicalis) will lead to adduction of the vocal folds, while by re-anastomosing the PoRLN with another recipient nerve (i.e. phrenic nerve) successful abduction of the vocal folds will also be achieved. However, in the human larynx the AnRLN contains all motor fibres and the distinction between adductor and abductor fibres within this nerve is impossible until the branch for each muscle is given off.[23] This scattered intrafascicular distribution of the motor fibres makes the selective re-anastomosis of the main trunk of the AnRLN with a recipient motor nerve problematic, resulting usually in laryngeal synkinesis. However, recently developed neuropeptides, such as the ORG 2766, seem to enhance the re-innervation process by stimulating axon sprouting, resulting in a more rapid and selective re-innervation pattern.[40]

Ideally, successful re-innervation of the transplanted larynx requires selective re-anastomosis of the PCA branch of the AnRLN with a motor nerve (i.e. phrenic nerve) and selective re-anastomosis of the remaining trunk of the AnRLN with another motor nerve (i.e. ansa cervicalis). SLN should also be anastomosed with a mixed nerve. However, the necessity to dissect, preserve and utilize the AnRLN branches for re-innervation of the transplanted larynx may be feasible for those patients who have lost the physiological function of the larynx through severe trauma but poses several concerns in patients with advanced laryngeal carcinoma, especially those with postcricoid, inferior extension and thyroid cartilage invasion. Unfortunately, these are probably patients who would otherwise certainly require total laryngectomy.

The innervation of the IA muscle is also a controversial topic. Many investigators in the past suggested that the IA muscle receives a double innervation, sensory from the InSLN and motor from the AnRLN.[13,32,41] However, consistent motor innervation through the InSLN has been confirmed recently by Sanders *et al.*,[18] although a similar suggestion has been found in the literature since 1930.[42] This dual motor innervation of the IA muscle could possibly enhance its selective re-innervation after laryngeal transplantation, through an anastomosis of the SLN with a mixed nerve. Furthermore, in 1997 Schweizer and Dorfl also demonstrated the presence of a small branch from the InSLN connecting the IA muscle with the PCA muscle (horizontal belly),[22] which may have important surgical implications, especially in laryngeal transplantation. In order to achieve successful abduction of the vocal folds any attempt to re-innervate the whole PCA muscle might also end up re-innervating some, or all, the IA muscle resulting in unwanted synkinesis. However, this problem may be avoided, as the re-anastomosis of the most PCA inferior branch (oblique belly) would in most cases avoid the PCA horizontal compartment with its strong inter-arytenoid connections.

Regarding the distribution of the ExSLN, Lemere was the first to suggest its contribution to the motor innervation of the TA muscle.[32] The existence of these motor axons was recently demonstrated by several investigators.[17,31,43] This dual innervation of the TA muscle may have important clinical implications in laryngeal transplantation since its re-innervation could also be enhanced through an anastomosis of the SLN with a mixed nerve, or more specifically through an anastomosis of the ExSLN with a motor nerve.

The development and testing of animal models that replicate human laryngeal function is one important component of current laryngeal research. Traditionally, investigators have used the dog as the principal animal model. The canine larynx approaches that of the human in size and shape, making the application of surgical techniques comparable in both species. The cat has been also used mainly for neurophysiological investigations of laryngeal function, especially the protective laryngeal closure.

However, laryngeal innervation in dogs/cats presents some differences from that in human. Thus, results and conclusions obtained from experiments on these animals can be applied to the human larynx only with the modifications based on the anatomical and physiological differences between these species. Additional disadvantages include the high cost and the tremendously labour-intensive protocols of peri-operative care required for maintaining these animals. The canine supraglottis has denser sensory innervation although with poorly developed fibres.[44] The significance of this pattern of sensory innervation regarding laryngeal transplantation is not clear, but if the sensitivity of the canine supralaryngeal mucosa is poorer the possibility of aspiration is larger in this animal model. Therefore, the successful outcome achieved in the canine transplanted larynx[45] allows us to be more optimistic in human laryngeal transplantation. Strome managed to avoid aspiration by successfully re-innervating his human transplanted larynx with sensory fibres through a re-anastomosis of the donor SLNs with the main trunks of the recipient SLNs.[3] In addition, the human InSLN is not purely sensory, as in the dog/cat, but also contains motor fibres for the IA muscle, that may enhance its motor innervation.

The fascicular organization of the human RLN is also more complex and variable and the distinction of motor and non-motor nerve fibres is impossible.[46–48] The communication between the SLN (or CrLN) and the RLN varies between these species. In man, this communication is achieved through Galen's anastomosis, the interarytenoid, the cricoid, the thyroarytenoid and the cricothyroid anastomosis while in dogs/cats through Galen's anastomosis, the PaRLN and the cricothyroid anastomosis (dogs). The implication of laryngeal anastomoses in the motor and sensory supply of the human larynx has been suggested in three parts: Galen's anastomosis as carriers of sensory proprioceptive fibres leading to different joints and muscles of the larynx, the interarytenoid as a carrier of sensory fibres to the posterior commissure and subglottis and a secondary source of motor innervation to the IA muscle and the cricothyroid anastomosis as a carrier of sensory fibres to the subglottis and as a secondary source of motor innervation to the TA muscle. The possible roles of the other two anastomoses (thyroarytenoid, cricoid anastomoses) remain to be established. Galen's anastomosis is also an important constant connection in the dog forming between the CaLN and the InCrLN. Its size is much larger than in other species (including man), approaching the size of the RLN. The PaRLN is also a constant connection in cats and probably in dogs but as it is absent in rats and humans, its role is not very well understood. As the cricothyroid anastomosis has not been demonstrated in cats, a secondary source of motor innervation through the ExCrLN to the TA muscle is not feasible. The PCA muscle of the dog/cat usually receives a single nerve branch from the RLN, while in most human larynges two, or even three, separate branches selectively innervate different parts of this muscle.[17] Unlike the canine PCA muscle, the human PCA muscle demonstrates only two distinct compartments, the oblique belly and the horizontal belly.[49] The oblique belly (innervated by the inferior PCA branches of the AnRLN) seems to be very effective at quickly opening the glottis during inspiration while the horizontal belly (innervated by the superior PCA branches of the AnRLN) helps in counterbalancing the pull of the CT muscle (via the vocal ligament) in high-pitched phonation. The concept of this selective innervation of the two human PCA compartments suggests that during laryngeal transplantation we could attempt to anastomose the inferior PCA branch with a motor nerve (in order to achieve vocal abduction) hoping that the horizontal PCA belly might receive innervation through its strong interarytenoid connections.

The innervation of the rat larynx has received less attention, although this animal model represents an attractive, inexpensive solution. Additionally, the most significant advantage is probably the more realistic dose response data obtained with xenobiotics in the rat compared with the more commonly used dog models.[50] However, the animal's small size, which calls for technically demanding surgical skills, is its main disadvantage. Anatomical studies have demonstrated that there is little or no proprioceptive laryngeal innervation and the lack of muscle spindles in the laryngeal muscles of the rat supports this statement.[38] To our knowledge, the presence of motor axons in the InCrLN for the IA muscle has not been determined yet, while the possibility for distinction of motor abductor-adductor fibres within the RLN is unknown. Although the CT, TA and LCA muscles have been found to receive double innervation from both the ExCrLN and the RLN, the precise innervation pattern of each laryngeal muscle remains unclear. However, for the CT muscle Kobler *et al.,* regarded the amount of crossover extremely small and probably of no functional significance.[39]

Detailed information on porcine anatomy is becoming increasingly important and several factors are interacting to produce this accelerated need. The availability of these animals makes them a convenient and accessible model. Additional advantages include vocalization, non-aggressive behaviour, hardiness and cleanliness. Furthermore, extensive studies have demonstrated the similarity of pig leukocyte subsets and their activities to those of the human confirming the value of the pig model for future immunological research.[51] The possibility of the pig as a potential donor for human xenografting may also be of relevance in the long term.[52] The innervation of the pig larynx is grossly similar to that of man and other species. However, despite the gross similarities in macroscopic anatomy, physiology and immunology between porcine and human airways, several aspects remain to be defined. The microscopic anatomy of the laryngeal nerves, especially the RLN, needs to be clarified in order to determine the intrafascicular organisation and distribution of these nerves. Furthermore, cross-innervation, multiple anastomoses and communications between the laryngeal branches have not been thoroughly investigated, hampering the routine use of this animal model in laryngeal research.

Therefore, considerable research concerning the microscopic anatomy and the neurophysiology of the laryngeal nerves is still required before laryngeal transplantation may be subjected to formal clinical trials. The establishment of the most suitable animal model in order to address these functional considerations is of obvious clinical value. Current investigations suggest that the pig could be the most appropriate of these models employed so far. However, substantial work on porcine neuroanatomy is urgently required.

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Dr P Stavroulaki takes responsibility for the integrity of the content of the paper.
Competing interests: None declared