LARYNGEAL REINNERVATION

IN THE CAT MODEL

Julie van Lith-Bijl

LARYNGEAL REINNERVATION IN THE CAT MODEL
Julie van Lith-Bijl

LARYNGEAL REINNERVATION

IN THE CAT MODEL

Publication of this thesis has been financially supported by Mediprof Medical Products, Entermed BV, Karl Storz Nederland BV, Smith & Nephew BV, Veenhuis Medical Audio BV, Stöpler BV, Medin Instruments, GlaxoWellcome BV, Astra pharmaceutica BV, Moduvice, Baxter-divisie Hyland/Immuno (producer of Tissucol[R]), Electro Medical Instruments, Hoechst Marion Roessel (producer of Telfast, Tavanic, Rulide, Sofradex), SmithKline Beecham Farma BV.

THELA · THESIS

ISBN: 90-9011908-6

VRIJE UNIVERSITEIT

LARYNGEAL REINNERVATION

IN THE CAT MODEL

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit te Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der geneeskunde
op woensdag 16 september 1998 om 15.45 uur
in het hoofdgebouw van de universiteit,
De Boelelaan 1105

door

Julie Tjitske van Lith-Bijl

geboren te Blantyre, Malawi

Promotor: prof.dr. H.F. Mahieu

Contents

Page

Chapter 1

Introduction

The larynx is located in the upper respiratory tract at the junction between the airway and the digestive tract. It serves to protect the airway from aspiration, to maintain optimal airway during respiration, and its vocal folds generate the vocal sound.

The intrinsic muscles of the larynx involved in these mechanisms are all innervated by the recurrent laryngeal nerve (RLN), with the exception of the cricothyroid muscles which are innervated by the superior laryngeal nerve. Paralysis of the RLN can lead to impairment of all three laryngeal functions due to the resultant immobility of the hemilarynx concerned. In unilateral paralysis phonatory problems predominate and can be accompanied by aspiration, especially in vagal nerve palsy. The airway usually remains sufficient. In bilateral RLN paralysis the airway is usually severely compromised, whereas phonation is usually less impaired. Swallowing problems are not obligatory but often occur.

Treatment, especially of bilateral RLN paralysis, has proved difficult as improvement or recovery of one function could only be obtained at the cost of the other functions. The treatment options other than permanent tracheotomy, usually comprised an anatomical widening of the airway, in bilateral RLN paralysis, at the cost of voice quality and increased risk of aspiration.

Physiological recovery of laryngeal functions by restoration of coordinated laryngeal mobility still presents a problem. Laryngeal reinnervation and laryngeal pacing are both methods which seek to bring about laryngeal mobility. Laryngeal pacing is associated with subsequent problems of implantation of electrodes and power source. Furthermore, there is still little data available on long-term nerve or muscle stimulation. This thesis will focus on laryngeal reinnervation as the method of choice to restore laryngeal function.

In order to understand the problems involved in restoring laryngeal mobility, the anatomy of the larynx and the RLN is first described. The physiological functioning of the larynx is summarized. Relevant to surgical reinnervation, the process of nerve damage, degeneration and regeneration is outlined.

Laryngeal reinnervation has been attempted in the past both in animal experiments and in the treatment of patients. Inconsistent and insufficient recovery of laryngeal function is generally reported.

With the recent performance of a human laryngeal transplantation by Strome, Cleveland, Ohio, USA (Birchall 1998) this subject has gained renewed interest. In laryngeal transplantation, laryngeal reinnervation should form an essential aspect, together with adequate revascularization and immunosuppression, as restoration of laryngeal function depends on appropriate reinnervation. During the transplantation performed by Strome reinnervation was attempted by reanastomosis of both superior laryngeal nerves and one RLN, the other RLN could not be identified. Although sensory reinnervaton may be established no adequate laryngeal motory restoration can be expected using these anastomoses. The procedure was solely performed to attain voice. Furthermore, the procedure was not performed in a cancer patient, but following crush injury to the larynx, thus avoiding the imminent problem of immunosuppression in cancer patients.

FUNCTIONAL ANATOMY OF THE LARYNX

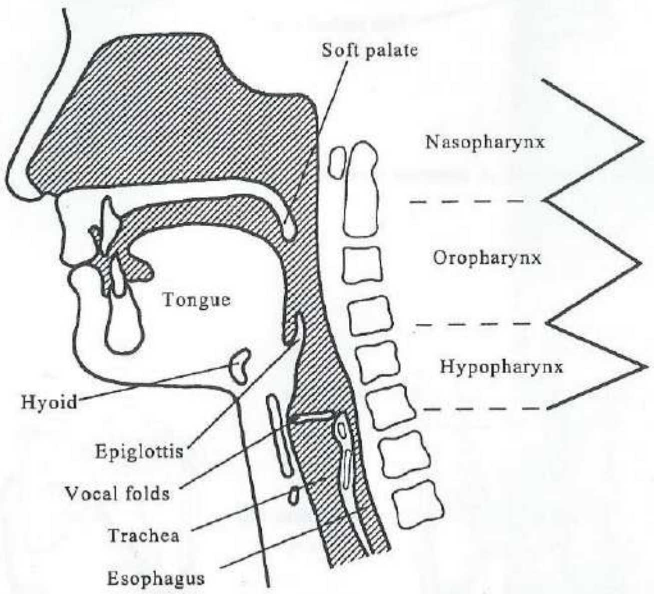


Figure 1.1 *The upper respiratory and digestive tract*

The upper respiratory tract consists of a number of connecting cavities, the nose, nasopharynx, oral cavity-oropharynx, hypopharynx, larynx and trachea (Figure 1.1). The larynx at the upper end of the trachea (Figure 1.2), consists of the annular cricoid cartilage, the thyroid cartilage and the paired arytenoid cartilages.

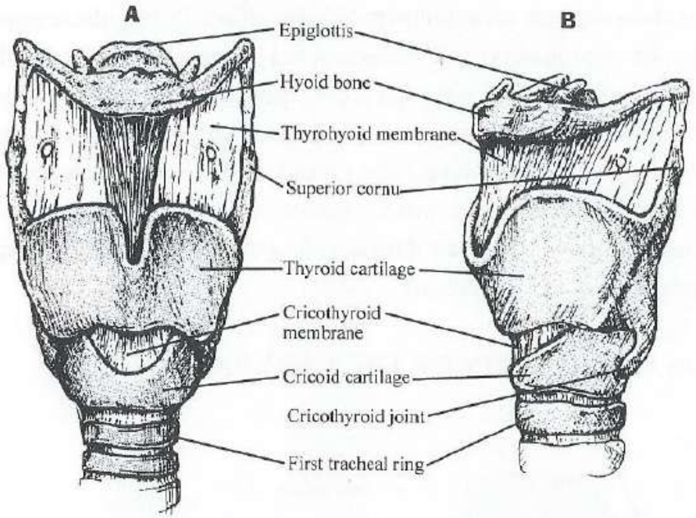


Figure 1.2 *Laryngeal framework, A, anterior view; B, lateral view*

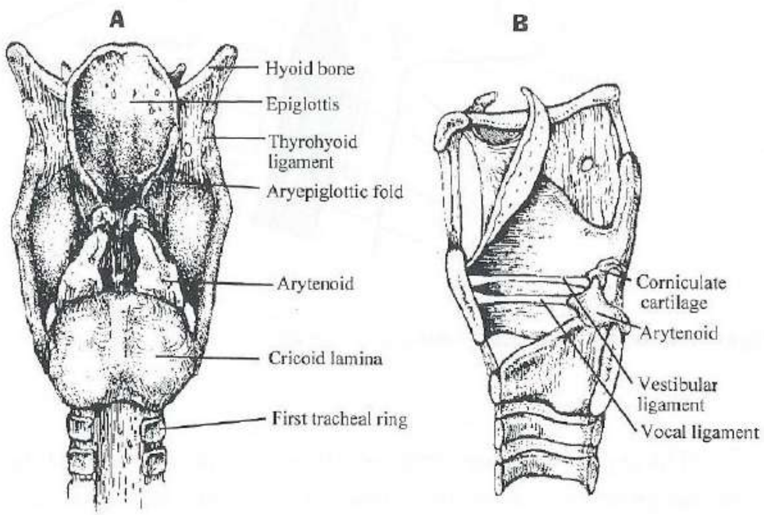


Figure 1.3 *Laryngeal framework, A, posterior view; B, sagittal view*

The thyroid can move with respect to the cricoid cartilage in two ways - by sliding forward, and by tilting forward and down. The arytenoid cartilages are seated on the upper rear edge of the cricoid cartilage (Figure 1.3) where they can be rotated (i) around their vertical axis, (ii) be moved toward or away from one another by sliding over the cricoid cartilage and (iii) be tilted forward around their horizontal axis.

The vocal folds are located between the anterior inner side of the thyroid cartilage, and the vocal processes of the arytenoids, and consist of folds of membranous tissue enclosing ligaments and muscles. The space between them is called the glottis (Figure 1.4). The ventricular folds are located just above the level of the glottis. The cranial part of the larynx is the epiglottis. The entrance of the larynx is lined by the aryepiglottic folds on either side which connect the epiglottis to the posterior part of the arytenoids. (Figure 1.4, Figure 1.5)

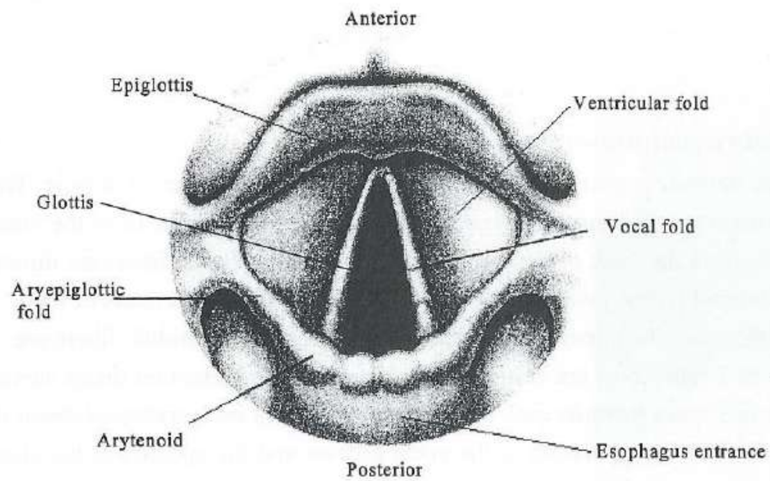


Figure 1.4 *Cranial view of vocal folds*

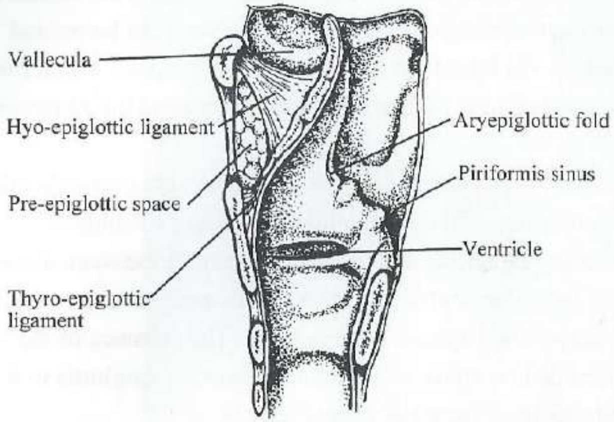


Figure 1.5 *Sagittal view of the larynx with intact mucosa*

Intrinsic laryngeal muscles

All the intrinsic muscles are paired except for the interarytenoid muscle. The **posterior cricoarytenoid muscle** (Figure 1.6) is the only true abductor of the vocal folds. It arises from the back of the cricoid lamina, from which the fibers are directed upward and laterally to be inserted onto the back of the muscular process of the ipsilateral arytenoid. The upper fibers are almost horizontal, the middle fibers are oblique and the lower fibers are vertical. Contraction of the horizontal fibers moves the muscular processes towards each other, thereby rotating each arytenoid about its own axis with resulting separation of the vocal process and the opening of the glottis. The vertical component draws the arytenoids downward on the sloping shoulder of the cricoid lamina in a lateral gliding motion, causing separation of the arytenoid without rotation. The oblique muscle fibers serve to stabilize the arytenoid during action of the other muscular components. Contraction of the muscular components occurs simultaneously, the lateral gliding displacement constitutes the greater portion of the total arytenoid movement during abduction.

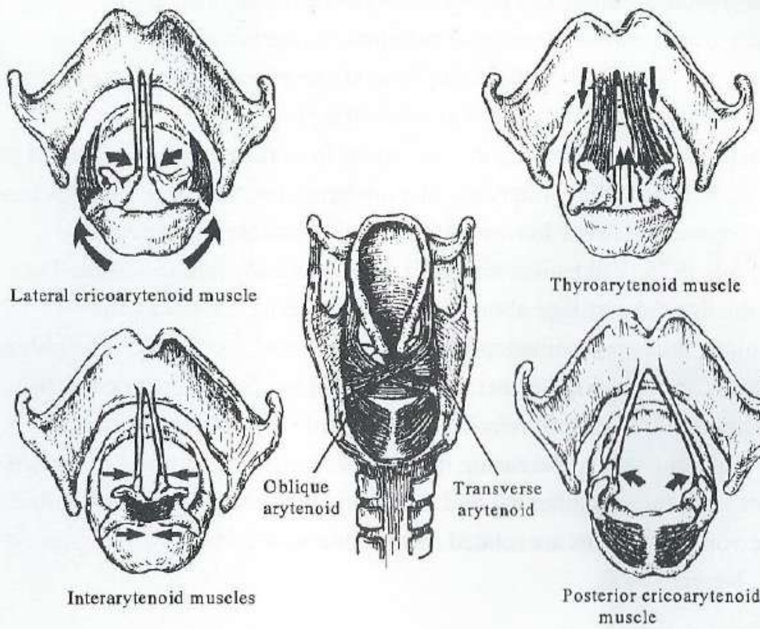


Figure 1.6 *Intrinsic laryngeal muscles*

The **lateral cricoarytenoid muscle** (Figure 1.6) arises from the upper border of the lateral part of the arch of the cricoid cartilage and is inserted into the anterior surface of the muscular process of the arytenoid cartilage. Contraction of the muscle draws the muscular process forward, causing approximation of the vocal processes by rotating the arytenoids medially.

The **thyroarytenoid muscle** (Figure 1.6) arises from the inside of the anterior angle made by the thyroid alae, and from the cricothyroid ligament below this. It forms a broad thin muscle situated lateral to the vocal ligaments and conus elasticus. The fibers pass backward laterally and superiorly to be inserted into the anterolateral surface of the arytenoid cartilage. The medial part of the muscle is thicker and forms a distinct bundle called the vocalis muscle. The fibers of the vocalis muscle are inserted into the vocal process of the arytenoid cartilage. Contraction of the thyroarytenoid muscle shortens and thickens the vocal folds.

The **interarytenoid muscle** (Figure 1.6) has two components: the paired pars obliqua and the single pars transversa, arising from the posterior surface and lateral border of one arytenoid cartilage and inserting into the corresponding parts of the contralateral arytenoid. Contraction draws the arytenoid cartilages up the sloping shoulders of the cricoid lamina, thereby approximating the arytenoid cartilages without rotation, in a so-called medial compression movement, closing the posterior glottic chink when the vocal folds are in an adducted position.

The **cricothyroid muscle** (Figure 1.7) arises from the anterior and lateral part of the cricoid arch, from which it fans out in a posterior direction, gaining attachment to the inferior cornua and lower border of the thyroid cartilage. There are two components (Fink 1975, Vilkman et al. 1987) the rectus and oblique bellies. The rectus rotates the thyroid cartilage about the horizontal axis, formed by the cricothyroid joints, thus approximating the cricoid arch and the thyroid. The oblique component causes anterior displacement of the thyroid relative to the cricoid, by ventrodorsal sliding of the cricoid relative to the thyroid. Contraction of the two bellies occurs simultaneously, increasing the distance between the vocal processes and the thyroid cartilage, thus lengthening and increasing the tension of the vocal folds. As a result the vocal processes are rotated inward thus also adducting the vocal folds (Arnold 1961, Negus 1947).

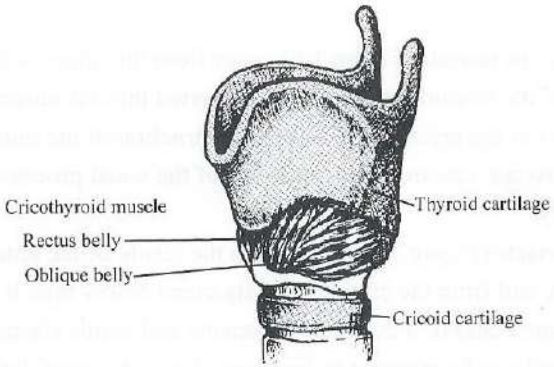


Figure 1.7 *The cricothyroid muscle*

In respiratory distress conditions, contraction of the cricothyroid muscle occurs together with contraction of the posterior cricoarytenoid muscle. By lengthening the

abducted vocal folds, the glottic space, the airway is enlarged (Suzuki et al. 1970, Horiuchi et al. 1978). In this condition the cricothyroid muscles have a subsidiary respiratory function.The intrinsic muscles interact in a coordinated fashion to abduct the vocal folds during inspiration and adduct them during swallowing, coughing and phonation. The collective force of contraction of the different muscles acts as a vector at a point in time, and determines the effective movement of the vocal fold. The only true abductor is the posterior cricoarytenoid muscle. All the other intrinsic muscles are adductors. The assumption that the activity of these muscle groups alternate to open and close the glottis is an oversimplification of the actual events. This assumption, however, serves as a useful model for attempting laryngeal function restoration.

Extrinsic laryngeal muscles

The extrinsic muscles of the larynx are constituted by the so-called strap muscles: the sternohyoid, thyrohyoid, sternothyroid and omohyoid muscles (Figure 1.8). The main function of these muscles is to adjust the position of the larynx in the neck as is most obvious, for example, when the larynx is elevated at the beginning of the pharyngeal phase of swallowing. These muscles, however, also have an accessory

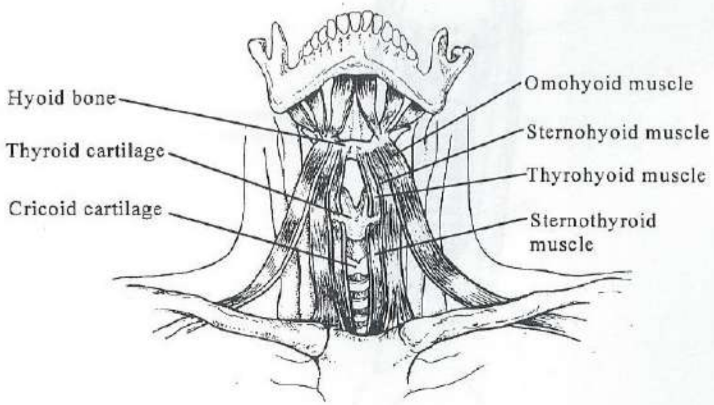


Figure 1.8 *Extrinsic laryngeal muscles*

respiratory function and are therefore also activated during respiratory distress. Their collective activity in this respect is most effective in non-calcified larynges, the cartilage having some degree of flexibility. This "pseudoactivity" of the vocal folds, should be distinguished from the true adductor and abductor activity of the larynx.

INNERVATION OF THE LARYNGEAL MUSCLES

The intrinsic laryngeal muscles with the exception of the cricothyroid muscle are all innervated by the RLN. The cricothyroid muscle is innervated by the external branch of the superior laryngeal nerve (SLN). The RLN and SLN are both branches of the vagus nerve, branching off along its descending cervical path. Before branching off, the RLN fibers form a separate bundle on the medial side, enclosed by a joint epineurium.

The left RLN (Figure 1.9) passes caudal to the aortic arch to then ascend along the oesophago-tracheal groove. The right RLN loops around the right subclavian artery to pass cranially along the esophageal groove to the larynx. The length of the

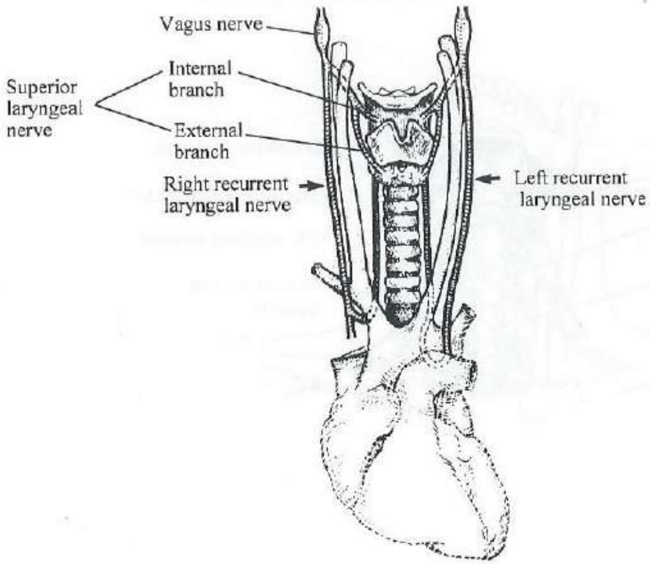


Figure 1.9 *Course of the recurrent and superior laryngeal nerves*

left RLN in the human exceeds the right RLN by about 10cm.

In the ascending part there are multiple sensory fibers branching from the RLN. One branch, usually branching extralaryngeally forms an anastomosis with the SLN, and is known as the ansa galeni. Its fibers are considered to be purely sensory in nature (Diamond et al. 1992). Wu and coworkers (1994) have described another communicating nerve branch in humans with evidence of a motor supply to the thyroarytenoid (TA) muscle arising from the SLN.

The RLN always proceeds posteromedially to the cricothyroid joint which is an important landmark for its identification. Intralaryngeally it divides into its motor branches. The posteromedial bundle runs to the posterior cricorytenoid muscle and the interarytenoid muscles. The anteromedial bundle (adductor branch) innervates the remaining intrinsic laryngeal muscles with the exception of the cricothyroid muscle. The alpha motor fibers reach the motor units from their parent nerve cell bodies in the nucleus ambiguus in the medulla oblongata. The interarytenoid muscle is the only muscle that receives bilateral innervation.

As mentioned earlier, the cricothyroid muscle is the only intrinsic laryngeal muscle which is not innervated by the RLN. It is innervated by the external branch of the SLN. The extrinsic laryngeal muscles including the omohyoid muscle are innervated by the ansa cervicalis branching off from the hypoglossal nerve.

THE INTRANEURAL STRUCTURE AND FUNCTION OF THE RLN

The main trunk of the RLN carries motor fibers to both the intrinsic laryngeal adductor and abductor muscles and contains 500-1000 myelinated nerve fibers. The ratio of adductor to abductor fibers is approximately 3:1. Besides efferent axons the RLN also carries many afferent axons. In the proximal RLN somato-motory fibers constitute only 30%, whereas just before its entry into the larynx the nerve is mainly motory, 80% of the fibers being somato-motory. The laryngeal motor nerves vary widely in diameter but most of them are between 6μm and 10μm (Gacek et al. 1976). Along its ascending path, the sensory fibers supplying the mucosa of the trachea and subglottic larynx, branching off from the RLN consist of relatively thin fibers. The RLN also has a relatively small number (2%) of large afferent axons, probably originating from the muscle spindles of the intrinsic laryngeal muscles (Suzuki et al. 1969). Finally, there are also efferent sympathetic and parasympathetic fibers with vasomotor and secretory functions (Crumley 1989).

The intraneural structure of the RLN forms no topographical separation of the abductor and adductor fibers. Sunderland and Swaney (1952) in their serial sections in humans found a constantly changing pattern with frequent communication between fascicles. The abductor and adductor fibers intertwine throughout the whole length of the nerve to only form separate bundles just before the RLN divides into the respective nerve abductor and adductor nerve branches. The abductor branch contains motor fibers supplying the PCA muscle. The adductor branch innervates the thyroarytenoid, vocalis, lateral cricoarytenoid muscles and interarytenoid muscles. The motor units of the adductor muscles are small and have 2-20 muscle fibers per motor unit and the muscle fibers mainly comprise muscle fibers with fast contraction times, 12-18ms (Hirose et al. 1969). The abductor muscle has much larger motor units with 200-250 muscle fibers per motor unit and has mainly fibers with slower contraction times, 30-50ms.

NEUROPHYSIOLOGY OF THE LARYNX

Neurophysiology of airway protective function

During the swallowing act, the larynx is elevated and moved anteriorly beneath the base of the tongue. The epiglottis is tilted covering the larynx, the passage of the bolus is propelled forward by contraction of the pharyngeal constrictor muscle and the upper esophageal sphincter opens. Simultaneous sphincteric closure of the upper airway occurs for protection of the airway.

In healthy subjects, sphinteric closure of the upper airway can be induced by bilateral SLN stimulation, which results in protective adduction of three muscular tiers within the laryngeal framework. The highest level of closure occurs at the aryepiglottic folds, which contain the superior most division of the thyroarytenoid muscle. With reflex contraction of these fibers, the aryepiglottic folds approximate to cover the superior inlet of the larynx. At this level the anterior gap is filled by the epiglottic tubercle and the posterior gap is filled by the arytenoid cartilages, completing the first of three sphincteric tiers of protection.

The second tier of protection occurs at the level of the ventricular folds. Laterally, along each fold are fibers of thyroarytenoid muscle that are capable of approximating the folds.

The third tier of protection occurs at the level of the true vocal folds, which are shelf like, with slightly upturned free edges. The inferior division of the thyroarytenoid muscle forms the bulk of each shelf, thus producing the potential for strong reflex closure. In conjunction with the passive valvular effect caused by the upturned edges of the vocal fold margin, the true vocal folds represent the strongest of the three barriers to aspiration.

Airway protective function of the larynx may be viewed neurophysiologically by examining the glottic closure reflex. This simple reflex produces protective laryngeal closure during deglutition. In this regard, electrostimulation of the internal branch of the SLN produces a low-threshold evoked action potential in the adductor branches of the RLN. In humans, the threshold of the adductor reflex measures 0.5V and has a latency of 25ms, indicating this to be a polysynaptic brainstem reflex, a view supported by appropriate calculations of latency measures (Sasaki et al. 1976, Suzuki et al. 1977). Unlike commonly used animal models, however, human subjects do not have a crossed adductor reflex; i.e. stimulation of the SLN only results in ipsilateral stimulation of adductor muscles and does not produce simultaneous action potentials in the contralateral adductor musculature. It is possible, therefore, that unilateral SLN injury in the human may result in failure of ipsilateral cord closure, a condition predisposing to aspiration despite the anatomic integrity of both RLNs.

SLN stimulation results in a variety of excitatory adductor responses but also exerts an inhibitory effect on the medullary inspiratory neurons. Not only does laryngeal abductor activity cease, but phrenic nerve activity is also inhibited, resulting in various degrees of apnea. Similar mechanisms occur during laryngospasm.

Neurophysiology of respiratory function

In 1949, Negus observed that the glottis opens a fraction of a second before air is drawn in by the descent of the diaphragm. In 1969, Suzuki and Kirchner established this activity as a direct effect of the medullary respiratory center, thereby opening the way to further understanding of laryngeal respiratory function. Having established that widening of the glottis occurs with rhythmic bursts of activity in the RLN, Suzuki and Kirchner (1969) then demonstrated that like phrenic nerve activity, this rhythmicity is accentuated by hypercapnia and ventilatory obstruction and depressed by hyperventilation and resultant hypocapnia.

From a purely structural perspective, because the true vocal folds move passively to obstruct the ingress of air to the lungs (Murakumi et al. 1972), active inspiratory abduction by phasic muscular contraction of the posterior cricoarytenoid muscle is essential to successful ventilation. With electromyographical techniques Sasaki and coworkers (1973) indeed proved that the phasic inspiratory abduction is synchronous with inspiration. Furthermore, the degree of abductor activity appears to vary directly with ventilatory resistance, disappearing entirely when inspiratory resistance is absent, only to return when resistance to ventilation is reestablished. Because selective deafferentation of the feline vagal nerve abolishes this response, it is believed that the afferent limb for the reflex modulation of phasic inspiratory abduction lies within the ascending vagus nerve (Fukuda et al. 1973). Receptors concerned with this reflex presumably lie within the thorax, although their exact nature and location are unknown. Pertinent to respiratory laryngeal function is the role of the cricothyroid muscle, known to lengthen and increase tension to the vocal folds. Neurophysiologic investigation demonstrates that this muscle contracts phasically with inspiration (Suzuki et al. 1970, Mathew et al. 1988). Its role in lengthening the vocal fold enhances the cross-sectional diameter by increasing its anteroposterior dimension. Furthermore, contraction of the cricothyroid muscles results in increased tension and stiffness of the vocal folds. This stiffness prevents passive adduction and decrease of glottal width induced by the ingress of air and the resulting Bernoulli effect. Therefore, it would appear that both the posterior cricoarytenoid and the cricothyroid muscles are driven by the medullary respiratory center, with the level of their activity modulated in eupneic breathing by afferent impulses originating in the chest. Posterior cricoarytenoid contraction increases the horizontal diameter of the glottic aperture, while its anteroposterior diameter is increased by phasic inspiratory contraction of the cricothyroid muscle. The cricothyroid muscle also exhibits expiratory activity and appears to play a major role in regulation of expiratory resistance and flow (Horiuchi et al. 1978). Effective adduction by contraction of the cricothyroid muscle only occurs when the posterior cricoarytenoid muscle relaxes, during the expiratory phase when the vocal folds move passively to the intermediate position (Murakumi et al. 1972).

Neurophysiology of phonation

Voice production is essentially the process of generating vibrating air, which is perceived as sound. The vibrating air is generated by the periodic opening and closing of the glottis by the passage of air between them. The frequency at which the vocal folds vibrate is the fundamental frequency of the tone produced. The periodic opening and closing of the glottis is regulated by a complex interaction of aerodynamic and myoelestic forces which is still not fully understood. But vocal fold vibration is a passive phenomenon. The aerodynamic theory of sound production finds support in the observation that the completely denervated larynx is capable of producing a fundamental tone, as is the cadaver larynx, when subglottic pressure is forcefully increased.

Although sound production itself may be considered a passive function, the onset, regulation and changes of phonation are not passive phenomena. Rather, vocal fold shaping and positioning are under direct neurophysiologic regulation. During phonation, the vocal folds are positioned near the midline by the lateral cricoarytenoid muscles. Additionally, the thyroarytenoid muscle provides finer isometric modification, reflected in fine shaping of the vocal folds. When the vocal folds are viewed in the frontal plane during phonation, the effect of shaping may be further appreciated. During the phonation of high-pitched tones, the folds seen on cross section appear thin, and during low pitches, the vocal folds appear to broaden considerably. Vocal fold tension and length are modified by contraction of the cricothyroid muscles.

The frequency of vibration depends on (1) vocal fold length, (2) vibratory mass, (3) antero-posterior tension, (4) stiffness of the vocal folds, and (5) subglottic pressure. As the true vocal folds tense and lengthen by the action of the cricothyroid muscles, pitch increases. Although cord lengthening alone would result in a reduction of pitch, this effect is offset by thinning and stiffening of the vocal fold produced by a combination of muscle contraction including the thyroarytenoid muscle, which increases the internal tension of the true vocal fold. Meanwhile, a counteraction occurs in the posterior cricoarytenoid muscle to stabilize the arytenoid. It also must be recognized that the activity of the extrinsic laryngeal muscles affects pitch by altering the spatial relation between the cricoid and the thyroid cartilages and may even have direct influence on the laryngeal framework especially in non-calcified larynges. Especially the sternothyroid muscle is thought to influence pitch in this way.

NERVE DAMAGE AND REGENERATION

The alpha motor nerve fibers innervating the motor units in the intrinsic laryngeal muscles reach them from their parent nerve cell bodies in the nucleus ambiguus in the medulla oblongata through the branches of the ipsilateral RLN. The cell bodies exchange biologic materials with their axon through several neuronal transport mechanisms. Both centripetal and centrifugal directions of axoplasmic flow have been demonstrated. Normally, much of this metabolism is concerned with production of neurotransmitter materials, such as cholineacetyltransferase and acetylcholinesterase (Ducker et al. 1977).

The alpha motor nerve fibers are myelinated. The characteristic structure is seen in Figure 1.10. The myelin sheath surrounds the axon, except at the location of the nodes of Ranvier. Immediately external to the myelin layer lies the cytoplasm of the Schwann cell, the so called sheath of Schwann. The Schwann cell and the layer immediately external to it, the endoneurium, form the endoneurial tube.

When a nerve is transected (axotomy) a biological signal is transmitted via centripetal axoplasmic flow to the cell body. A marked metabolic transformation takes place in the cell body. The production of substances concerned with excitation and depolarization, such as acetylcholinesterase is decreased dramatically. Concurrently, RNA synthetase and glucose-6-phosphatase dehydrogenase levels are increased. These substances are important in protein and lipid synthesis for regeneration. Wallerian degeneration takes place distal to the injury site consisting of dissolution of the axon and its myelin sheath. The Schwann cell and endoneurium undergo changes but persist as a contracted empty endoneurial tube, expectantly awaiting the subsequent arrival of a regenerating axon sprout. The segment proximal to the injury

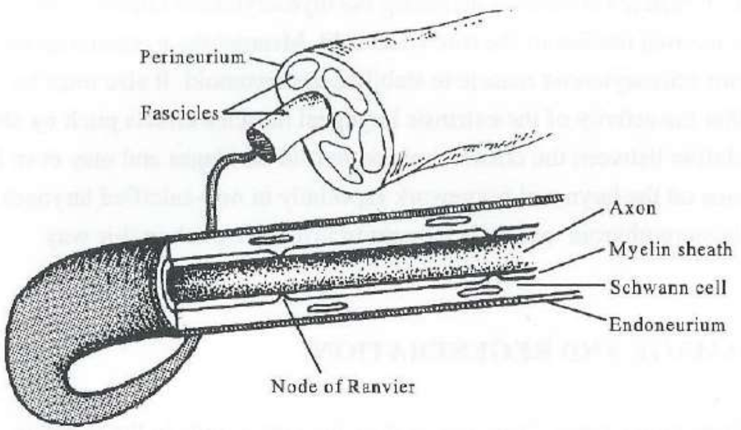


Figure 1.10 *Alpha motor nerve fiber (longitudinal cross section). The Schwann cell and endoneurium form the endoneurial tube*

site undergoes degeneration over a length of about 1cm, the remainder stays intact. Between the first and third weeks, each individual axon will show tiny axon buds to probe the periphery. Each axon sprout has the opportunity to enter any vacant endoneurial tube. Each endoneurial tube will accept only one sprout. Through the multiple sprouting mechanism it is possible for one axon to innervate several endoneurial tubes. Other axons may push to the peripheral end of the nerve, but may not achieve their goal without the protective and inductive function of the endoneurial tube.

After an axon attains an endoneurial tube it biochemically induces the Schwann cell to reproduce a myelin layer. When the axon reaches the peripheral end of the nerve, neuromuscular continuity is established by reformation of the motor end plate. Nerve conduction and muscular function do not appear until a further period of time has elapsed. This period is believed to represent the time in which the cell body reconverts its metabolic machinery to produce those substances required for normal nerve function, while diminishing those materials solely concerned with regeneration of the axon.

Axonal regeneration is enhanced by neurotrophic substances such as gangliosides and melanocortins. Melanocortins are neuropeptides and are related to adrenocorticotrophic hormone (ACTH) and melanin stimulating hormone (MSH) and have similar molecular structures. The influence of a variety of ACTH-related substances have been tested on stimulation of nerve regeneration. Org 2766, an ACTH4-9 analogue, has been found to have a potent neurotrophic activity. Earlier studies have shown Org 2766 to enhance axon regeneration probably by stimulating axon sprouting (Dekker 1987a and b, Tonnaer et al. 1992).

Furthermore, muscle denervation results in local production of substances which appear to increase their susceptibility to reinnervation. These substances include "neurocletin" (Hoffman 1950) and "nerve growth factor substance" (Spiro 1978) and are believed to stimulate axon sprouting.

LARYNGEAL REINNERVATION

Surgical reinnervation of the larynx encompasses a surgical technique using nerve anastomoses, implantation of a nerve-end or nerve muscle pedicle into a recipient muscle to allow regenerating axons to grow into and reinnervate the laryngeal muscles.

Laryngeal reinnervation can be either nonselective or selective. Nonselective reinnervation comprises anastomosis to the mainstem of the RLN leading to reinnervation of both abductor and adductor muscle groups. In selective reinnervation the abductor and/or adductor muscles are separately reinnervated.

Nonselective reinnervation

By anastomosing either the RLN proximal stump or a foreign donor nerve to the mainstem of the RLN the regenerating axons can grow along the adductor as well as the abductor branches and will simultaneously reinnervate both muscle groups.

RLN-repair

The first reports of laryngeal reinnervation were all cases in which reanastomosis of the cut proximal and distal stumps of the RLN was performed. In 1909, Horsley reported the first successful vocal fold reinnervation, describing complete return of function following RLN reanastomosis in a 40-year-old female patient following a gunshot injury. This led to a number of investigations with conflicting results. In 1926, Blalock and coworkers reported experimental repair of the RLNs in dogs. They described successful results suturing the distal cut end of one RLN into a slit on the side of the contralateral RLN. In 1928, Lahey reported successful repair of the RLN in a patient, with subsequent limited restoration of function. McCall and coworkers (1946) also reported successful repair in patients.

Colledge and coworkers (1927) were the first to describe paradoxical glottic movements of expiratory abduction and inspiratory adduction following RLN repair. Thereafter, study reports involving direct RLN repair repeatedly described unsuccessful restoration of function (Clerf 1937, Hoover 1953, Capps 1958).

Introduction of electromyography (EMG) allowed further evaluation of these "failures". Sirhibodhi and coworkers (1963) performed a careful electromyographical study following regeneration of repaired RLN nerves in dogs. They found EMG activity but no meaningful function. They proposed the theory of aberrant regeneration of adductor and abductor fibers to inappropriate motor end plates, a phenomenon already well documented in other nerves, especially the facial nerve.

In 1968, Gordon and McCabe reported in dogs that accurate alignment without twisting of the nerve stumps would permit physiological regeneration. However, only return of adduction was reported and it is not unlikely that this adduction was a result of cricothyroid muscle action, unrelated to the RLN reanastomosis. Similar findings were reported by Boles and coworkers (1969).

Doyle and coworkers (1967) reanastomosed the RLN in 4 dogs and evaluated the reinnervation using electrical stimulation. They concluded that reanastomosis was successful on the basis of electrical stimulation although no spontaneous function was observed. Reanastomosis of the cut RLN in 2 patients was also reported (Doyle et al. 1967). In one patient both RLNs had been transected in traumatic injury. Reanastomosis in the acute phase resulted in 80% recovery of laryngeal mobility on one side and complete immobility in the paramedian position on the contralateral side, 10 months post-injury. The patient had an excellent voice and had no complaints of the airway even during physical exertion. The second patient had reanastomosis in the acute phase after thyroid surgery. Eighty percent recovery of movement was reported, a normal voice and no respiratory difficulty.

Murakumi and Kirchner (1971) found no purposeful motion after RLN reanastomosis and regeneration of the RLN in dogs. They divided the adductor branch within the larynx and found that abduction would then occur with inspiration. Stimulation of the SLN, normally evoking glottic reflex closure, even resulted in paradoxical abduction of the vocal fold. This again supported the importance of the role of synkinesis in the impaired recovery of laryngeal function mobility.

In summary, the consensus over the years has been that RLN repairs are incapable of producing cyclic abduction or adduction (Tashiro 1972, Mu et al. 1990, Nahm et al. 1993).

Anastomosis of foreign nerves to the RLN mainstem

Attempts to reinnervate the extralaryngeal distal part of the RLN main trunk with foreign nerves have also been attempted, using the vagus nerve, the phrenic nerve and the ansa cervicalis.

The vagus nerve was used by a number of investigators but none of them reported recovery of laryngeal function (Ballance 1924, Colledge 1925, Barnes 1928). In 1967, Doyle anastomosed a split vagus nerve to the mainstem of the RLN in 8 dogs. Although he claimed success in 4 dogs, none had recovery of spontaneous function.

Recovery of function was tested by electrical stimulation which was carried to tetanic spasm. If the cord had an identical position as during electrical stimulation before surgery, the recovery was said to be successful. Murakumi and coworkers (1971) similarly performed nonselective reinnervation with the vagus nerve in 2 dogs and reported the same purposeless motion as the dogs that underwent RLN-reanastomosis.

The phrenic nerve has been used as early as 1924 by Ballance in a patient resulting in excessive, but purposeless motion of the vocal fold. Repeated studies, (Barnes et al. 1927, Barnes 1928) also reported no adequate return of function. Murakumi and coworkers (1971) studied the same technique in dogs and found paradoxical adduction during inspiration until the adductor branch was transected intralaryngeally. Thereafter, adequate abduction occurred during each inspiration. In 1974, Iwamura reported 10-60% recovery of abduction after phrenic-RLN anastomosis without transection of the adductor branch in dogs.

The ansa cervicalis was suggested as a possible donor nerve for laryngeal paralysis as early as 1924 (Frazier). Frazier and coworkers (1926) reported improved phonation and relief of dyspnea using this technique in 5 of 10 patients with bilateral vocal fold paralysis. Ballance (1924) reported failure in one patient with end-to-end anastomosis of the ansa hypoglossi to the RLN.

Much later, Crumley and coworkers (1986, 1991), extensively used this technique in patients with unilateral vocal fold paralysis. No active mobility was achieved but patients did develop muscle tonus in the adductor musculature and improvement of the voice.

In summary, nonselective reinnervation, therefore, irrespective of the foreign nerve used, will always lead to laryngeal synkinesis as the regenerating axons grow to both abductor and adductor muscle groups. Therefore, no effective return of function can be expected. If a donor nerve with a very weak activity pattern, such as the ansa cervicalis, is used, the synkinesis results in immobility of the vocal fold with recovery of muscle tonus, without adverse purposeless motion. Although no adductor function recovery occurs, recovery of the muscle tonus alone can lead to voice improvement as Crumley demonstrated (Crumley 1986, 1991).

Selective reinnervation

Selective reinnervation is accomplished by reinnervating the adductor and abductor muscle groups separately using different "foreign" nerve transfers for each muscle group. The foreign nerves are usually chosen to match the activity required in the respective muscle groups. In general three different techniques have been applied to achieve selective laryngeal reinnervation: neural implantation, nerve-muscle pedicle implantation and selective nerve anastomosis.

Neural implantation

Neural implantation comprises implantation of the proximal stump of a cut motory nerve into a denervated muscle in order to reinnervate the muscle by muscular neurotization and formation of new motor end plates (MEP's). Several donor nerves have been used for implantation in the posterior cricoarytenoid (PCA) muscle: the mainstem of the RLN, the phrenic nerve and the sympathetic cervical trunk.

Doyle (1967) implanted the proximal stump of the RLN in the PCA muscle in 7 dogs. He evaluated function only by electrical stimulation. He reported that in 3 dogs the abducted position of the vocal fold was the same as before surgery when carried to tetanic spasm and concluded this to be a good functional result.

Fex (1970) performed this technique in 23 cats of which only in 8 cats reinnervation of the PCA muscle with partial to normal restoration of abductor function was achieved; in the remaining 15 cats avulsion of the nerve implant occurred. Doyle and coworkers (1993) implanted the phrenic nerve in 12 cats; in 9 functional abductor reinnervation occurred, and in 3 cats failure was caused by detachment of the nerve. Taggart (1971) implanted the phrenic nerve into the PCA muscle in 6 dogs, 3 of which also resulted in avulsion of the phrenic nerve. Brondbo and coworkers, in 1987, used a nerve graft to anastomose the phrenic nerve to achieve adequate length and implanted a stump into the PCA muscle in dogs. After a reinnervation period of seven months, he reported poor functional results, because of laryngeal synkinesis. Coinciding adductor reinnervation was found but the origin of this inappropriate reinnervation remained unclear.

Morledge, in 1973, achieved good results implanting the phrenic nerve in the PCA muscle in 12 dogs. He evaluated the effect of a delay period between denervation and reinnervation surgery and concluded that the extent of abduction varied inversely with the length of the delay period.

The sympathetic cervical trunk which is known to have a very weak respiratory activity has also been implanted into the PCA muscle in 4 dogs (Jacobs et al. 1990). No vocal fold abduction was seen during quiet respiration, slight abduction during hyperpnea.

In summary, neural implantation in the PCA muscle with a nerve with an appropriate activity pattern such as the phrenic nerve, can result in functional abductor reinnervation. To achieve reinnervation with this method it is essential that the motory nerve has length in excess, or that a free nerve graft is used, to avoid avulsion during swallowing. Using the RLN to reinnervate the PCA muscle, restoration of laryngeal function is doomed to fail, as the mixed activity pattern of the nerve is not appropriate.

Nerve-muscle pedicle technique

The technique encompasses transfer of an intact donor nerve in continuity with a small block of the muscle it serves (together referred to as a nerve-muscle pedicle (NMP)), to the recipient muscle. The NMP is buried into a pocket created by an incision in the fascia of the recipient muscle and fixed in situ.

Tucker first examined the NMP technique in dogs (Tucker et al. 1970, 1971) as a method for reinnervation of the larynx in laryngeal transplantation. He excised the adductor and abductor nerve branches in continuity with a small block of muscle, proved that the vocal fold was denervated and then replaced the NMPs. He was able to demonstrate reinnervation and restoration of function as early as 2 weeks after surgery. Tucker speculated on two possible mechanisms of reinnervation using this technique. Firstly, reinnervation could result by "spillover" of depolarizing motor units within the NMP to surrounding muscle fibers of the recipient muscle. Tucker assumed that with activity induced by spillover of depolarizing motor units from muscle fiber to muscle fiber, formation of new neuromuscular junctions would not be necessary. Secondly, he proposed occurrence of axon regeneration from the NMP into the recipient muscle followed by reinnervation of denervated muscle end plates.

Later Tucker applied the NMP technique in order to restore laryngeal abduction. Having demonstrated success in dogs using the sternohyoid branch of the ansa cervicalis to reinnervate the PCA muscle (Hengerer et al. 1973, Lyons et al. 1974), Tucker treated patients with bilateral vocal fold paralysis using the sternohyoid branch (Tucker 1975) and later the omohyoid branch (Tucker 1978) of the ansa cervicalis. He reported return of spontaneous inspiratory abduction in most patients and some were even able to have their tracheostomy tube removed. No EMG was performed, however, to prove denervation prior to surgery or to prove reinnervation after surgery.

Crumley tested the ansa cervicalis NMP in 5 dogs (Crumley 1982b). Apparent vocal fold abduction was seen during hyperpnea, from airway obstruction, in all dogs, but was abolished by SLN transection or detachment of sternothyroid muscle, suggesting abductory activity resulting from activity of the cricothyroid or extrinsic laryngeal musculature. None of the nerves in the NMP's were electrically excitable. Crumley, therefore, concluded that the NMP procedure does not result in

reinnervation of the PCA muscle. Rice (1983a) also failed to show any evidence of reinnervation using an NMP with the ansa cervicalis in 7 dogs.

In summary, reinnervation using the NMP technique is perhaps feasible, and most likely by neurotization of the recipient muscle from the NMP, provided the recipient muscle has recently been denervated, as an innervated or subclinically reinnervated muscle is not susceptible to reinnervation.

Selective surgical reinnervation by nerve anastomoses

Selective surgical reinnervation can basically be performed in two different ways. First, a foreign nerve can be directly anastomosed to the abductor or adductor branch, if necessary in combination with a free nerve graft to cover the distance. Second, by anastomosis to the mainstem of the RLN and then either directing all the nerve branches toward the target muscle or ligating the other nerve branches.

Abductor reinnervation

For selective abductor reinnervation the following nerves have been used, the split-vagus nerve, the ansa cervicalis, a split-phrenic nerve graft and the phrenic nerve.

Miehlke (1974) anastomosed a split-vagus nerve directly to the abductor branch of the RLN in 6 dogs. By using a split-vagus nerve he was able to cover the distance to the abductor branch. Reinnervation of the PCA muscle was evaluated after 10-12 months and compared to the unoperated side. Synchronous abduction of both vocal folds was found. Miehlke did not specify the degree of abduction during inspiration of the operated side nor whether abduction was found in all dogs. Laryngeal mobility during swallowing or coughing, elicited in the experimental condition by stimulation of the internal branch of the SLN (the afferent component of the glottic closure reflex) was not investigated.

Miehlke performed the split-vagus nerve anastomosis to the abductor branch ("vagus recurrent bypass anastomosis") in a patient in 1972 with bilateral vocal fold paralysis after a thyroidectomy four months earlier (Miehlke 1974). Ten weeks after the surgical reinnervation an abduction movement was observed during inspiration. After six months the vocal fold had resumed immobility in the intermediate position, no abduction was seen on phonation. Mielhke concluded that the result was comparable with a successfully performed laterofixation.

Attali and coworkers (1988) anastomosed the ansa cervicalis, sternothyroid branch to the abductor branch to reinnervate the PCA muscle in 15 dogs. An immobile vocal fold in abduction position was seen in 14 dogs and an inspiratory polyphasic

EMG pattern was seen in 12 of these dogs. The abduction allowed adequate airflow during quiet respiration and appeared to be the result of increased tonus of the PCA muscle throughout the respiratory cycle. The ansa cervicalis normally innervates the strap muscles and is only activated during forced inspiration, the strap muscles being active mainly in respiratory support. Rice (1983b) applied the same technique in 4 dogs. After four months inspiratory abduction was seen on the reinnervated side varying between 50 and 70% of the normal side. Transection of the ansa cervicalis resulted in loss of abduction.

Crumley (1982b) selectively reinnervated the PCA muscle using a split-phrenic nerve graft procedure in 10 dogs. Splitting the phrenic nerve and using a length of 15cm free nerve graft, harvested from the ansa cervicalis, enabled the distance to be covered to connect the split-phrenic nerve to the abductor branch. The adductor branch was ligated. Five dogs showed successful reinnervation, and good function during respiratory distress, only 2 showed good abductor function during quiet respiration. Avulsion of the nerve from the PCA muscle was the cause of failure in 4 dogs and from the phrenic nerve in 1. Furthermore, regeneration of RLN axons through the ligated proximal adductor branch accounted for simultaneous inappropriate reinnervation of the adductors and poor functional results.

The split-phrenic nerve graft technique has been applied in four patients with bilateral laryngeal paralysis (Crumley 1983). In all cases the paralysis had existed for over 24 months. In none of the patients inspiratory abduction could be observed two years after the reinnervation. One patient claimed a subjective improvement of stridor and dyspnea during physical exertion. Crumley assumed that avulsion of the nerve had occurred in 2 patients and that in the remaining 2 cases, pre-existent subclinical reinnervation was the reason for failure. Subclinical reinnervation had been evident during electrical stimulation of the RLN in these 2 cases before the reinnervation procedure, and denervation was not performed at the time of reinnervation surgery. The subclinically (re)innervated PCA muscle was therefore not susceptible to reinnervation.

The phrenic nerve was also used by Rice (1982) to selectively reinnervate the PCA muscle in 8 dogs. He anastomosed the phrenic nerve to the RLN mainstem and tied off the adductor branch. Ten to 24 weeks after the surgical reinnervation, in 6 of the 8 dogs excellent abduction on inspiration was reported, and 1 dog showed 50% abduction as compared with the normal side.

The best results have been achieved using the phrenic nerve in a cat model by Baldiserra and coworkers (1986). They anastomosed the cranial root of the phrenic nerve to the mainstem of the RLN and directed the branches toward the PCA muscle in 9 cats. Forty-five to 60 days after surgical reinnervation inspiratory abduction was seen which was equal to the normal side during quiet respiration in all cats. In 2 cats the abduction of the reinnervated side even exceeded that on the normal side. EMG recordings revealed a typical phrenic nerve activity pattern which started 30 to 40 milliseconds later than in the PCA muscle, slowly increasing at the onset of inspiration, and a slight decrease followed by an abrupt end of activity, at the end of inspiration. The normal PCA muscle, typically, almost immediately, reaches its maximal activity at the onset of inspiration and slowly decreases towards the end. The resting EMG baseline activity, which is present during expiration in the normal PCA muscle, was absent in the reinnervated PCA muscle. Electrical stimulation proved regeneration of the nerve. Histology with horse radish peroxidase staining and its retrograde transport established reinnervation by the phrenic nerve.

In summary, excellent restoration of abductor function can be achieved using the phrenic nerve to selectively reinnervate the PCA muscle, especially in the cat model in which spontaneous laryngeal mobility could be observed. The phrenic nerve is the most appropriate substitute for the abductor fibers as its inspiratory activity pattern closely resembles that of the normal PCA muscle. The split-vagus nerve with its mixed activity pattern is unlikely to bring about appropriate abductor function. The ansa cervicalis only has inspiratory activity during respiratory distress and thus cannot be expected to bring about inspiratory abduction during normal breathing.

Adductor reinnervation

Attempts have been made to selectively reinnervate the adductor by anastomosing either the SLN or the ansa cervicalis to the adductor branch.

Rice (1982) anastomosed the external branch of the SLN to the adductor branch in a group of 10 dogs. Nine dogs were evaluated, of which 4 showed excellent glottic closure during cough or whine. Two dogs showed an immobile vocal fold in the median position, for which no explanation was available. In 1 dog abduction only occurred on electrical stimulation, in 1 dog the anastomosis had broken down, and in 1 no cause of failure was found.

Zheng and coworkers (1996a) investigated anastomosing the ansa cervicalis to the adductor branch in 9 dogs. In 7 of the 9 dogs good to excellent abduction was described during whining. In 1 dog the vocal fold was immobile in the intermediate position and in the other the vocal folds were immobile in the medial position.

This technique was then applied in 8 patients (Zheng 1996b) with unilateral vocal fold paralysis. EMG was recorded in 5 patients and proved that reinnervation had taken place in all 5 cases. No effective movement was seen of the paralyzed vocal fold, but objective and subjective improvement of the voice quality, loudness and pitch was demonstrated in all the patients, as well as improvement of acoustical parameters (jitter, shimmer and normalized noise energy).

Selective reinnervation of the adductor muscles is mainly of interest for unilateral vocal fold paralysis after which phonatory problems predominate and to a lesser extent aspiration problems may occur. No optimal substitute is available for the adductor fibers which are active during phonation, swallowing, and coughing (reflex glottic closure). Using the SLN to reinnervate the adductors means sacrificing the main accessory adductor, Crumley (1984) has described this as "robbing Peter to pay Paul", which would be difficult to advocate. The ansa cervicalis brings about muscle tonus but no active adduction which nevertheless can result in voice improvement (Zheng 1996b).

Separate selective abductor and adductor reinnervation

Iwamura (1974) anastomosed the phrenic nerve to the RLN mainstem and simultaneously performed anastomosis of a split-vagus nerve directly to the adductor branch which had been ligated just after its division from the RLN mainstem. In all 6 dogs tested, reinnervation of both the thyroarytenoid and the PCA muscles occurred as confirmed by return of normal EMG patterns after an average period of 2.2 to 2.6 months. After a period of 12-14 months the degree of adductor function restoration was described as 50-100% and abductor function 10-60% compared to the unoperated side. In 2 dogs an adductor spasm was seen at the beginning of each inspiration for a fraction of a second. This paradoxical mobility was ascribed to adductor reinnervation by abductor fibers partly comprising the split-vagus nerve which are active at the onset of inspiration. Iwamura explained the poorer restoration of abductor function compared with the adductor function to the longer distance the regenerating phrenic nerve axons had to grow to reach the PCA muscle.

In 1984, Crumley combined selective abductor reinnervation with the phrenic nerve and a free nerve graft in dogs, with a simultaneous selective adductor reinnervation. Adductor reinnervation was attempted by suturing the ansa cervicalis (2 dogs) , the external branch of the SLN (3 dogs) or the proximal RLN stump to the adductor branch of the RLN (4 dogs). One year after the reinnervation in all except 1 dog abduction of the vocal fold occurred on electrical stimulation of the anastomosed nerve. The degree of spontaneous abduction was comparable with normal phonation and coughing. In 4 of the 9 dogs spontaneous inspiratory abduction occurred. In these cases electrical stimulation of the phrenic nerve or along the graft section also resulted in abduction. In 1 dog in which the adductor was reinnervated with the RLN and the PCA muscle with a phrenic nerve graft, the abductor was reinnervated by the phrenic nerve as proved by electrical stimulation. In 1 of the dogs with an RLN anastomosis spontaneous inspiratory abduction was observed but neither the abductor nor the adductor branch was stimulable. The spontaneous abduction disappeared by transection of the RLN. This led Crumley to believe that the RLN has a strong proclivity to reinnervate intrinsic laryngeal muscles. The RLN had a biological advantage over the phrenic nerve and graft as the original blood supply had been left intact and the regenerating axons had a shorter distance to cover before reaching the denervated muscle. Once the PCA muscle has been reinnervated by the RLN it is no longer susceptible to reinnervation by the phrenic nerve.

Marie and coworkers (1989) reported results of selective abductor and adductor reinnervation with the phrenic nerve and ansa cervicalis, respectively, in dogs. Seven dogs were evaluated 3 and 8 months after the surgical reinnervation. Functional rehabilitation of both abductor and adductor functions occurred in only 1 dog. Only abduction was restored in 5 dogs. The most important reason for failure to restore abductor function was said to be axonal escape from the anastomosis. Histological findings indeed revealed axonal escape. The regenerated phrenic nerve axons, therefore, may well have also reinnervated the antagonistic adductors, resulting in synkinesis.

In summary, separate selective adductor and abductor reinnervation has been realized in the animal model, in which various different donor nerves have been used. The best abductor function results have been achieved using the phrenic nerve. For selective adductor reinnervation there is no foreign nerve available which is optimally suited. Use of the ansa cervicalis with its weak activity pattern during normal breathing, has been shown to restore muscle tone with voice improvement. The loss of normal ansa cervicalis function has no great disadvantages and altogether at this time appears to be the best choice as a donor nerve for restoraton of adductor function.

AIMS OF THE STUDY

Despite the numerous reports on experimental laryngeal reinnervation, apparently no satisfactory procedure has as yet been developed that can be applied in humans to adequately restore laryngeal function. Regeneration after reanastomosis of a transected RLN, as well as any method of nonselective reinnervation, have usually been shown to lead to laryngeal synkinesis, with impaired laryngeal function. Separate selective reinnervation of the opposing abductor and adductor muscle groups appears to be a promising option to restore laryngeal reinnervation as this method, at least theoretically, prevents the adverse effects of synkinesis.

The continuing quest for laryngeal transplantation over the years, and the concurrent necessity to restore laryngeal function, has stimulated us to further investigate the possibilities of laryngeal reinnervation. In the relatively rare cases of bilateral RLN paralysis or injury, there is, at present, no good solution for the combined problems of a compromised airway, phonatory disorders and increased risk of aspiration. Experimental research on laryngeal reinnervation may lead to a physiologic restoration of these laryngeal functions.

In the more common cases of unilateral RLN paralysis, phonatory problems predominate. Although satisfactory treatment methods are available, laryngeal reinnervation may also offer an alternative treatment to improve voice quality. Therefore, experimental studies are mandatory and we chose to perform such a study in animal model.

The aims of the study were

- to investigate restoration of laryngeal function after different types of nerve injury
- to confirm the feasibility of selective laryngeal reinnervation with respect to abductor and adductor function
- to determine the influence of delay of selective laryngeal reinnervation on the functional outcome
- to determine the influence of nerve regeneration enhancement by administration of Org 2766 on functional outcome
- to develop a management protocol for laryngeal nerve injury on the basis of animal experiments and clinical experience.

We chose to study laryngeal reinnervation in a cat model. Cats can be anesthetized satisfactorily while breathing spontaneously using ketamine chloride and xylazine hydrochloride without interfering with spontaneous inspiratory laryngeal movement and glottic reflex closure. Therefore, laryngeal function can be accurately evaluated without having to perform electrical stimulation, which does not reflect spontaneous activity. Furthermore, the feline RLN and its branches are large enough to be anastomosed under the operating microscope.

In **Chapter 2** laryngeal function recovery obtained after crush injury or nonselective reanastomosis after nerve transection is described.

In **Chapter 3** selective reinnervation methods were investigated since nonselective reanastomosis did not result in adequate function restoration, as was anticipated. As respiratory function is most important in bilateral RLN paralysis our first efforts were directed toward abductor restoration. Selective abductor reinnervation was performed with a phrenic nerve transfer as this nerve has an inspiratory activity pattern similar to the abductor nerve fibers. The surgical procedure was performed in a similar way as described by Baldiserra and coworkers (1986) in an effort to confirm their excellent results. The results of an integrated double-blind placebo controlled pilot study to examine nerve regeneration enhancement by administration of the neuropeptide Org 2766, are also presented in this chapter.

In **Chapter 4** selective adductor restoration was investigated. The adductor function is more difficult to substitute with a foreign nerve than the abductor function. For phonation, restoration of muscle tone by, for example, reinnervation with the ansa cervicalis has been shown to improve voice quality. In this chapter, selective adductor reinnervation with the ansa cervicalis was combined with separate selective abductor reinnervation with the phrenic nerve.

Chapter 5. In clinical practice usually some time has elapsed before surgical reinnervation can be taken into consideration after RLN injury. The nerve injury may not immediately be apparent or a tracheotomy may have been performed in the early stage to secure an adequate airway. Therefore, the feasibility of selective abductor reinnervation after a delay period between onset of paralysis and surgical reinnervation is of interest for its application in humans. A 9-month delay was chosen before selective abductor reinnervation was performed with the phrenic nerve.

Chapter 6. In order to develop a management protocol for acute unilateral RLN injury, the results of spontaneous abduction and glottic reflex closure were evaluated and compared after crush, nonselective reanastomosis, selective adductor reinnervation in the acute phase and transection of the RLN without reconstruction. The clinical results of 2 patient histories in which the RLN was accidentally transected during thyroid surgery and reanastomosed in the acute phase are described. An algorithm for management of acute injury to the RLN was proposed considering these results and results reported in the literature.

Chapter 7. Experimental laryngeal transplantation is a recurring issue in laryngological research. Recently, the first human laryngeal transplantation has been performed in a patient who lost his laryngeal functions due to trauma. The transplantation was performed by Strome (Birchall 1998). A number of factors play a crucial role in success of laryngeal transplantation: revascularization, immunosuppression and reinnervation. Revascularization has proved feasible especially with present microsurgical techniques. Most of the patients who would be eligible for laryngeal transplantation are cancer patients. Immunosuppression is known to increase the risk of metastatic disease and is still a major issue in research. The most difficult problem to solve, however, remains the problem of adequate reinnervation in order to restore laryngeal function. This has not been attempted by Strome but should be considered one of the major issues in contemplating this type of surgery. The results obtained in our studies are reviewed in this respect.

Chapter 8. Throughout the study a double-blind placebo controlled trial was integrated to test the effect of Org 2766, a neuropeptide which has been demonstrated to enhance nerve regeneration after crush injury in rats (Tonnaer et al. 1992). The histological and functional results with regard to the Org 2766 or placebo treatment are considered.

Chapter 2

Laryngeal Abductor Function after Recurrent Laryngeal Nerve Injury in Cats

Julie T van Lith-Bijl (MD), Hans F Mahieu (MD,PhD),
Robin J Stolk, Jeroen ADM Tonnaer (PhD),
Cees Groenhout (MD), Pierre NM Konings (PhD).

Archives of Otolaryngology, Head & Neck Surgery 1996;122:393-396

Abstract

Objective To determine the influence of severity of neural injury of the recurrent laryngeal nerve on recovery of laryngeal abductor function and the importance of synkinesis.

Design The recovery of laryngeal abductor function was studied in 30 cats after crushing (second degree injury) or transection followed by reanastomosis (fifth degree injury) of the recurrent laryngeal nerve, with a reinnervation period of 10 weeks.

Main outcome measures Recovery of laryngeal abductor function was evaluated by videolaryngoscopy of spontaneous laryngeal abduction during respiration and by electromyography of the posterior cricoarytenoid and vocalis muscles. Neural lesions were applied unilaterally, and recovery of laryngeal function was compared with the contralateral unimpaired hemilarynx. Reinnervation was confirmed by histologic examination.

Results After the recurrent laryngeal nerve was crushed, laryngeal abductor function was similar to normal after a 10 week reinnervation period in 19 of the 20 cats; after reanastomosis, no notable recovery of laryngeal abduction resulted in any of 10 cats. Electromyographic recordings disclosed synkinesis after reanastomosis and recovery of normal activity patterns after crush injuries.

Conclusions Severity of neural injury to the recurrent laryngeal nerve influences the recovery of laryngeal abductor function. Damage to the endoneurium leads to misdirection of regenerating axons, inappropriate reinnervation and synkinesis. No effective laryngeal function can then be expected.

Introduction

Recovery of laryngeal function after a recurrent laryngeal nerve (RLN) injury is an important clinical issue because of the highly coordinated activity required for proper functioning of respiration, swallowing and phonation at the crossroads of airway and digestive tract.

Poor recovery of laryngeal function, with permanent immobility, or restricted mobility of the hemilarynx, often has been observed after surgical reinnervation attempts (Hiroto et al. 1968, Tashiro 1972, Siribodhi et al. 1963, Dedo 1971). Misdirected reinnervation is considered to be the cause of this poor functional result (Hiroto et al. 1968, Tashiro 1972, Siribodhi et al. 1963, Crumley 1989). Misdirected reinnervation is a phenomenon that occurs after nerve damage. Regenerating nerve fibers sprout and may grow along paths to other denervated muscles than to the original target muscle. For the RLN, misdirection of regenerating axons is facilitated by the intraneural topography. Abductor and adductor axons are intertwined and divide into the subsequent branches only intralaryngeally, close to their target muscles (Sunderland et al. 1952). Misdirected regeneration of these axons leads to inappropriate reinnervation of opposing muscle groups. The neural activity, which is adequate, thus causes simultaneous contraction (synkinesis) of adductors and abductors, resulting in inadequate mobility, immobility, or paradoxical movement of the larynx. Laryngeal paradoxical movement implies adduction during inspiration, abduction during phonation, or both.

The presence of synkinetic activity can be established by electromyography (EMG) as shown in 1963 by Siribodhi and coworkers The incidence of synkinesis after RLN injury in humans is unknown (Crumley 1979) and, in clinical practice, is probably grossly underestimated; especially in cases of total immobility after vocal cord paralysis. In animal experimental reinnervation studies (Siribodhi et al. 1963, Dedo 1971, Crumley 1979) synkinesis varies between 66% and 88%.

To explain the occurrence of synkinesis in the facial nerve, a correlation between synkinesis and severity of nerve injury has been suggested (Crumley 1979). Sunderland (1952) classified peripheral nerve injuries into five degrees of severity. A first-degree injury corresponds to neuropraxia. The axon and its endoneurial tube (consisting of sheath of Schwann and endoneurium) retain continuity through the injury site. Axoplasmic flow continues; only neural conduction is lost. Complete recovery of normal function occurs, often within hours.

A second-degree injury involves loss of axonal continuity but the endoneurium remains intact. Wallerian degeneration occurs, and the distal nerve does not respond to stimulus 72 hours after injury. The regenerating axon stays within the endoneurial tube and thus is directed toward the original target muscle. Nerves subjected to first- and second-degree injuries usually recover their normal function.

In a third-degree injury, axonal and endoneurial discontinuity occurs. In fourth- and fifth-degree injuries disruption of the fascicular arrangement of the nerve trunk and transection of the whole nerve trunk occur, respectively.

In third-, fourth- and fifth-degree injuries the regenerating axon has no confining conduit to direct it to its appropriate muscle. Multiple sprouting occurs with sprouts randomly entering the distal endoneurial tubes, which are unlikely to be the original tube. Misdirected axons cause reinnervation of inappropriate muscles, and synkinesis results. To determine the influence of the severity of a neural lesion to the RLN on recovery of laryngeal abductor function and the importance of synkinesis, we studied the laryngeal abductor function after crushing the nerve (second-degree injury) and after transection followed by reanastomosis (fifth-degree injury). We specifically evaluated recovery of the abductor function. Restoration of abductor function is clinically important because of the respiratory problems caused by a bilateral RLN injury.

Materials and methods

Surgical method

Thirty female cats (aged, 6 months, weight range, 2200g to 2800g) were anesthetized with ketamine chloride (20mg/kg intramuscularly) and xylazine hydrochloride (0.5mg/kg subcutaneously), allowing spontaneous respiration. A midline incision of the neck was performed. Pretracheal muscles were separated in the midline and the proximal part of the trachea was exposed. The right RLN was then identified.

In 20 cats, a crush injury of the right RLN was induced. At a distance of 2.0cm from the cricothyroid joint, the nerve was crushed for 30 seconds with hemostatic forceps with grooved jaws (Halstead No. 02.401.12), by closing the forceps for three clicks (Tonnaer et al. 1992). Immediately after the nerve was crushed, the distal point of the crush injury was marked with a suture in the perineurium.

In 10 cats the right RLN was transected at a distance of 2.0cm from the cricothyroid joint. The ends were then reapproximated and reanastomosed with a 10-0 microsuture (Ethilon, Ethicon Inc Somerville NJ) and fixed with fibrin glue (Tissucol, Austrian Hemoderivates GES m.b.h., Vienna, Austria). An extra suture was placed adjacent to the site to facilitate reidentification.

Assessment of laryngeal abductor function

To assess laryngeal abductor function, videolaryngoscopy and EMG, were performed, using the same anesthesia that was used for the surgical procedure. Electromyography was performed by transorally introduced hooked wire electrodes in the left and right posterior cricoarytenoid (PCA) muscle, and in the left and right vocalis muscles (pars medialis of the thyroarytenoid muscle). An EMG type MS6 (Medelec, Old Walking England) was used. Respiratory monitoring was performed simultaneously, using a custom-made impedance plethysmograph, registering chest movement.

Laryngeal abductor function was assessed before and following the creation of the neural lesion, and thereafter, weekly, during the 10-week follow-up period. The time at which first signs of mobility and EMG activity were seen was recorded. Laryngeal abductor function was evaluated after 10 weeks. To eliminate influence of the cricothyroid muscles or of the extrinsic laryngeal muscles on the abduction and adduction of the right vocal fold, these muscles were severed bilaterally before the final assessment.

The operated-on right side was compared with the normal left side. The spontaneous abduction was classified as *poor*, no effective or slight abduction on inspiration; *limited*, abduction on inspiration half or less than half of the maximal abduction of the left side; *adequate*, abduction on inspiration more than half but slightly less than the maximal abduction of the left side; *good*, abduction on inspiration synchronous with and equal to or more than the maximal abduction of the left side.

Electromyographic recordings of the right vocalis and right PCA muscles were analyzed considering signs of an inspiratory activity pattern. After final assessment and electrical stimulation of the right RLN proximal to the lesion, the animals were killed.

Histology

Histologic examination of the nerve anastomosis was performed to obtain histologic proof of reinnervation. The right RLN was fixed in situ in the anesthetized animal using a cacodylate-glutaraldehyde solution and was then resected (Tonnaer 1992). After postfixation in the same solution, the tissue was embedded in epoxy resin and 1-μm-thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histologic evaluation was performed 0.5cm distal to the anastomosis. By computed screening of a 500μm band across the widest nerve diameter, the axon count (axons per square millimeter) was estimated.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney *U* test to compare the results of the crush injury and reanastomosis groups.

Results

Preoperatively, all cats showed symmetric, spontaneous laryngeal abduction on inspiration. Immediately postoperatively, the right vocal cord was immobile in all cases. Denervation of the right RLN after the crush injury and transection was confirmed by EMG inactivity in the right vocalis muscle and the right PCA in all cases.

Crush injury

A first sign of mobility after crush injury of the right RLN was seen with videolaryngoscopy on average after 2.5 weeks (range, 2 to 4 weeks) and comprised a slight trembling of the right vocal cord. Electromyographically, signs of reinnervation were recorded in the right vocalis muscle at an average of 2.4 weeks (range, 2 to 3 weeks) and in the right PCA at an average of 2.9 weeks (range, 2 to 4 weeks). This difference was not statistically significant (p=0.6).

Good recovery of abductor function was found in 16 of the 20 cats; in two of these cats, the maximal abduction on the right side even exceeded that on the normal left side. In three cats the abduction was adequate. In one cat the abduction was limited, which seemed to be attributable to a developing ankylosis of the cricoarytenoid joint that was diagnosed on palpation of the arytenoid. None of the cats

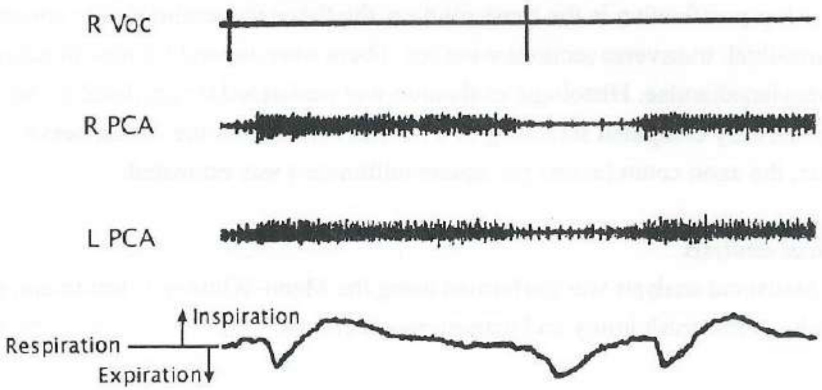


Figure 2.1 *Top, Electromyography (2cm/s) 10 weeks after crush lesion to the right recurrent laryngeal nerve. Synkinesis is absent (no sign of inspiratory activity in the right vocalis muscle). PCA indicates posterior cricoarytenoid muscle. Bottom, The respiratory cycle. Simultaneous videolaryngoscopy (not shown) demonstrated normal abduction.*

showed poor recovery of abduction, nor were there signs of paradoxical mobility.

Normal inspiratory activity was recorded in the right PCA muscle of all 20 cats. The right vocalis muscle showed no activity on inspiration in 19 cats (Figure 2.1). In one cat minor simultaneous inspiratory activity was recorded in the right vocalis muscle simultaneous with that in the right and left PCA. The activity in the right vocalis muscle consisted of the action potential of a single motor unit increasing in frequency at inspiration and decreasing during expiration; a rich recruitment pattern was recorded during inspiration in the right PCA. No inspiratory activity was found in the left vocalis muscles of any of the cats.

Electrical stimulation of the right RLN was performed in 19 cats. In all cases, stimulation resulted in a laryngeal response, visible during videolaryngoscopy and EMG recording. In 1 cat, electrical stimulation could not be performed because iatrogenic damage to the RLN had occurred during reexploration of the anastomosis site.

Histological evaluation was performed in specimens acquired from 19 of the 20 cats after the crush injury. The remaining specimen had to be excluded from evaluation because of technical inconsistencies during the post-fixation procedure. Regenerated axons were seen in all 19 specimens, the median axon count was 3778 axons per square millimeter.

RLN-reanastomosis

After reanastomosis, first signs of mobility of the right hemilarynx were seen with videolaryngoscopy at an average of 3.9 weeks (range, 3 to 6 weeks). The mobility, as after crush injury, comprised a slight trembling movement of the right vocal cord. First signs of EMG activity were recorded in the right vocalis and right PCA muscles at an average of 3.8 weeks (range, 3 to 5 weeks) after surgery.

All 10 cats showed poor abduction during inspiration 10 weeks after reanastomosis. No signs of paradoxical movements during inspiration were observed. Simultaneous inspiratory activity in the right PCA and the right vocalis muscles was recorded in all 10 cats. The EMG activity was characterized by a rich recruitment pattern during inspiration simultaneously in the right vocalis muscles and right and left PCA muscles (Figure 2.2). No inspiratory activity was found in the left vocalis muscles of any of the cats.

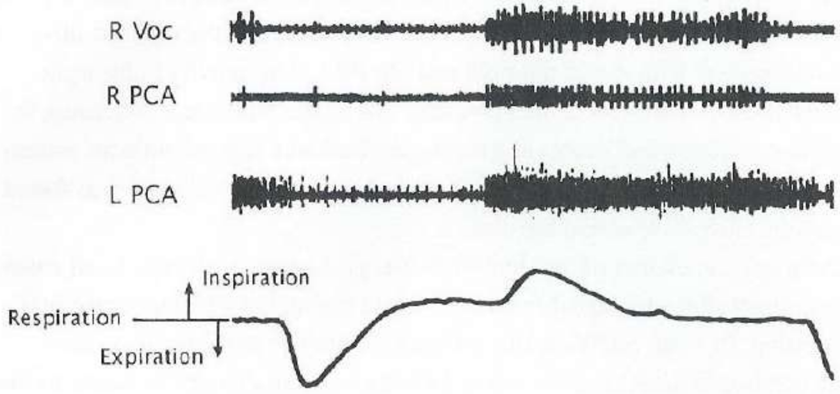


Figure 2.2 *Top, Electromyography (2cm/s) 10 weeks after neurorrhaphy of the right recurrent laryngeal nerve. Simultaneous inspiratory activity in the right vocalis (RVoc) and right posterior cricoarytenoid (PCA) muscles indicate synkinesis. Bottom, The respiratory cycle. Simultaneous videolaryngoscopy (not shown) demonstrated almost total immobility of the right vocal fold.*

Electrical stimulation of the right RLN was performed in 9 of the 10 cats. In all cases, stimulation resulted in a laryngeal response, visible during videolaryngoscopy and EMG recording. In the remaining case, iatrogenic damage to the RLN occurred during reexploration of the anastomosis site, so electrical stimulation could not be performed.

Histologic evaluation was performed in specimens acquired from 9 of the 10 cats. Because of a technical inconsistency during the postfixation procedure, the remaining specimen was excluded from evaluation. Regenerated axons were seen in all specimens; the median axon count was 3339 axons per square millimeter.

Statistical analysis

First signs of return of mobility were significantly earlier after crush injury than after reanastomosis ($p<0.001$). First EMG signs of reinnervation in the right PCA muscle were seen significantly later, after reanastomosis than after crush injury ($p<0.01$). Reinnervation in the right vocalis muscle also appeared significantly later in the reanastomosis group than in the crush group ($p<0.001$). After 10 weeks a

gnificantly better abductor function resulted after the crush injury than after the anastomosis ($p<0.001$). Synkinesis occurred in significantly more cats after anastomosis than after crush injury ($p<0.001$). Histologic examination showed the ımber of regenerated axons per square millimeter of the RLN, distal to the lesion, 10 eeks after crush injury or reanastomosis to be similar.

iscussion

Severity of injury to the RLN influences the recovery of laryngeal abductor ınction after reinnervation. This is not a new observation. Boles and Fritzell (1969) vestigated crush injury and reanastomosis of the RLN in dogs and reached the same onclusion. However, in that study the influence of the strap muscles and the icothyroid muscles was not excluded, as it was in our study. Especially in non-lcified larynges, the activity of the strap muscles and cricothyroid muscles can result a significant "pseudomobility" of the vocal cords (Fink et al. 1956, Kotby et al. 970, Suzuki et al. 1970). Although Boles and Fritzell (1969) did not exclude the ossibility of pseudomobility, their study showed that reanastomosis leads to sfunction based on synkinesis.

This observation has had little effect on the views about laryngeal uromuscular function and reinnervation. In 1989, Crumley again pointed out the portance of synkinesis in laryngologic practice. Meanwhile, many unsuccessful forts had been made to reinnervate the larynx by making anastomoses with several rves to the distal RLN stump.

Although unrecognized, these failures undoubtedly resulted from synkinesis. nkinesis is probably the most common condition of a so-called laryngeal palsy that s been immobile for more than 6 months, as those who regularly perform laryngeal MGs know. This, however, changed the concept of laryngeal reinnervation. pparently, the aim should not only be to achieve reinnervation, but also to achieve innervation without synkinesis. This can be achieved only by separate reinnervation adductor and abductor muscle groups (selective reinnervation).

Our study shows the uselessness of RLN-reanastomosis for recovery of nction. Recovery of tonus is the most one can expect from such a procedure, but ter crush injury, return of normal laryngeal abductor function is the rule. Only one se of crush injury showed minimal signs of synkinesis on the EMG recording, thout apparent interference with recovery of laryngeal function. In principle, ushing the nerve may preserve the endoneurial tubes but in practice sometimes may use more severe damage, thus allowing sprouting and sporadic inappropriate reinnervation to occur.

The practical consequences of this study and other studies are that in surgical or traumatic cases in which the RLN has been injured, stretched, or clamped without losing continuity, it is worthwhile to wait for spontaneous recovery. When the continuity has been lost, reanastomosis or any other nonselective reinnervation procedure may at best result in a restoration of muscle tonus. In cases with a bilateral loss of continuity of the RLN, good recovery of function, can be achieved only by a separate selective reinnervation of abductor and adductor muscle groups (Baldiserra et al. 1986, Mahieu et al. 1993).

Chapter 3

Selective Laryngeal Abductor Reinnervation in Cats using a Phrenic Nerve Transfer and Org 2766

Hans F Mahieu (MD, PhD), Julie T van Lith-Bijl (MD),
Cees Groenhout (MD), Jeroen ADM Tonnaer (PhD),
Peter de Wilde (MD).

Archives of Otolaryngology, Head & Neck Surgery 1993;119:772-776

Abstract

Reinnervation of the recurrent laryngeal nerve following nerve injury often leads to laryngeal synkinesis. Selective reinnervation of adductor and abductor muscles might be able to avoid synkinesis. This study presents the results of selective abductor reinnervation in cats, using a phrenic nerve transfer to the recurrent laryngeal nerve and directing all reinnervating axons towards the abductor muscle. Simultaneously a blind, placebo controlled, pilot study was performed to evaluate the capacity of Org 2766, administered subcutaneously (25μg/kg/48hours), to facilitate reinnervation by stimulation of axon sprouting. Reinnervation surgery was performed in 10 cats. Postoperative evaluation included videolaryngoscopy, electromyography, histological examination and quantification of reinnervating axons. Nine cats could be evaluated, of which 8 demonstrated electromyographic and laryngoscopic activity as soon as 6 weeks following surgery. The 1 cat showing no abduction, was found to have an inadequate nerve anastomosis and was marked as a surgical failure. After 10 weeks near-normal or more than normal abduction was seen in the 8 cats and histological proof of reinnervation was obtained in 7 of them; 1 cat could not be evaluated histologically due to unsuccessful fixation. Although no conclusive evidence was obtained concerning the effect of Org 2766, the tendencies found warrant further experiments with this compound on laryngeal reinnervation.

Introduction

The recurrent laryngeal nerve (RLN) innervates laryngeal adductor as well as laryngeal abductor muscle groups. Injury of the RLN causing partial or total denervation results in impairment of the complex coordinated interaction of adductor and abductor muscle groups.

The intertwined architecture of adductor and abductor axons within the RLN (Sunderland et al. 1952) facilitates misdirection of reinnervating axons. Adductor axons can thus inappropriately reinnervate abductor muscles and vice versa.

The uncoordinated simultaneous contraction of laryngeal adductor and abductor muscles following this misdirection of axons will result in a mobility disorder of the affected hemilarynx. This mobility disorder may present as a reduced but otherwise adequate mobility, a paradoxical mobility (adduction during inspiration and abduction during phonation), or an immobility of the hemilarynx. Also, combinations of these mobility disorders can occur. This so-called laryngeal synkinesis is the reason why laryngeal function often remains impaired despite the fact that reinnervation has actually occurred as shown in Chapter 2 and in other

studies (Crumley 1989).

The literature on surgical laryngeal reinnervation is extensive, but the majority is concerned with either RLN reanastomosis or anastomosis of another nerve to the RLN main stem. In all these situations laryngeal synkinesis will develop, as was already noted as early as 1963 by Siribodhi and coworkers.

Therefore, surgical attempts to achieve functional laryngeal reinnervation should be directed towards selective appropriate reinnervation and prevention of synkinesis. This can be achieved by reinnervating the adductor and abductor muscle groups separately using different nerve transfers for each muscle group (Rice 1983, Crumley 1983, Crumley 1984, Baldiserra et al. 1986, Attali et al. 1988, Marie et al. 1989). The donor nerves will have to have activity patterns comparable to the laryngeal function which they have to restore. For restoration of abduction a respiratory-dependent activity during inspiration is required, as is found in the phrenic nerve (PN).

The aim of the present study was to test this concept of selective laryngeal reinnervation for the abductor function. We chose to start with attempting to accomplish restoration of abductor function because of the predominant respiratory problems with which a bilateral RLN injury presents itself. Good restoration of abductor function has been described by Baldiserra and coworkers (1986) in cats using a PN tranfer.

The anatomical situation of the PN in the cat differs from that in humans in two important respects. First, the cat has two distinguishable PN roots, which unite in the PN proper in the lower part of the neck, just cranially of the thorax aperture. This situation enables the transfer of only one of these roots, leaving at least part of the PN innervation to the diaphragm intact. Second, the length of either of the PN roots in the cat is not sufficient to anastomose them directly to the abductor branch of the RLN. Therefore, Baldiserra and coworkers (1986) anastomosed the PN root with the main stem of the RLN. The adductor branch of the RLN was then severed, so that reinnervating PN axons could not reach the adductor muscles. Thus, a selective reinnervation of the abductor muscle was achieved, provided that no axonal escape towards the adductors occurred.

An interesting recent development in reinnervation studies is the experimental use of neuropeptides which are thought to enhance the reinnervation process by stimulating axon sprouting (Strand et al. 1980, Verhaagen et al. 1987). The peptide often used for this purpose is a corticotrophin analogue known as Org 2766 (Met(O_2)-Glu-His-Phe-D-Lys-Phe) (Organon International BV, Oss, the Netherlands). Several studies, mostly involving crush lesions and reanastomosis performed on the sciatic nerve in rats, have demonstrated a favorable effect of these neuropeptides on function restoration (De Koning et al. 1987, Dekker et al. 1987, Tonnaer et al. 1992). However, beneficial effects have also been reported following nerve transection and microsurgical repair in rats (Edwards et al. 1986).

To investigate the possibility of stimulating laryngeal reinnervation with Org 2766, we integrated a blind, placebo-controlled treatment in our study.

Materials and methods

Surgical method

Ten female cats (aged 6 months to 1 year) were anesthetized with ketamine chloride (20mg/kg intramuscularly) and xylazine hydrochlorde (0.5mg/kg subcutaneously), allowing spontaneous respiration. Laryngeal function was assessed preoperatively and immediately postoperatively by videolaryngoscopic monitoring and hooked wire electromyography (EMG) of the left and right posterior cricoarytenoid (PCA) muscles. Respiratory monitoring was performed simultaneously using a custom-made impedance plethysmography, registering chest movement.

A modification of the surgical technique described by Baldiserra and coworkers (1986) was used. The right RLN was identified. A small part of the thyroid cartilage was resected to expose the adductor and abductor nerve branches. The abductor branch was preserved and the adductor branch was severed; its proximal stump was buried in the right PCA muscle using a 10-0 microsuture (Ethilon, Ethicon Inc Somerville NJ) and fixed with fibrin glue (Tissucol, Austrian Hemoderivates GES m.b.h., Vienna, Austria). To exclude any influence of the ansa galeni, although unlikely because of its sensory nature (Diamond et al. 1992), it was also severed and its proximal stump buried in the right PCA muscle. The two roots of the right PN were identified. The uppermost PN root was resected just before it joined the other root. The right RLN was severed 2.0cm from the cricothyroid joint and the distal RLN stump was anastomosed to the proximal PN stump using a 10-0 microsuture and fibrin glue. Thus, all reinnervating PN axons were directed towards the PCA muscle, even those that followed the path of the adductor branch or the ansa galeni (Figure 3.1).

Org 2766

All cats were injected subcutaneously with 0.1ml/kg every 48 hours during 1 month, starting at the time of reinnervation surgery; half of them (group A) were injected with solution A, the other half (group B) with solution B. At the time of the

study the investigators were unaware which solution contained Org 2766, 250mg/L, and which solution was placebo. Animals treated with the active ingredient thus received Org 2766 at a dose of 25µg/kg every 48hours.

Follow-up

Laryngeal function was assessed every 2 weeks using the same anesthesia that was used for the surgical procedure, allowing spontaneous respiration. The assessment was performed by videolaryngoscopic monitoring and hooked wire EMG of the left and right PCA muscle. Respiratory monitoring was performed simultaneously using impedance plethysmography. After 10 weeks the animals were killed following assessment of laryngeal function in rest, during respiratory distress and during electrical stimulation of the PN. The respiratory distress condition was achieved by performing a tracheotomy and occluding the tracheostoma for 1 minute. To eliminate any influence of the strap muscles or the cricothyroid muscles on the abduction and

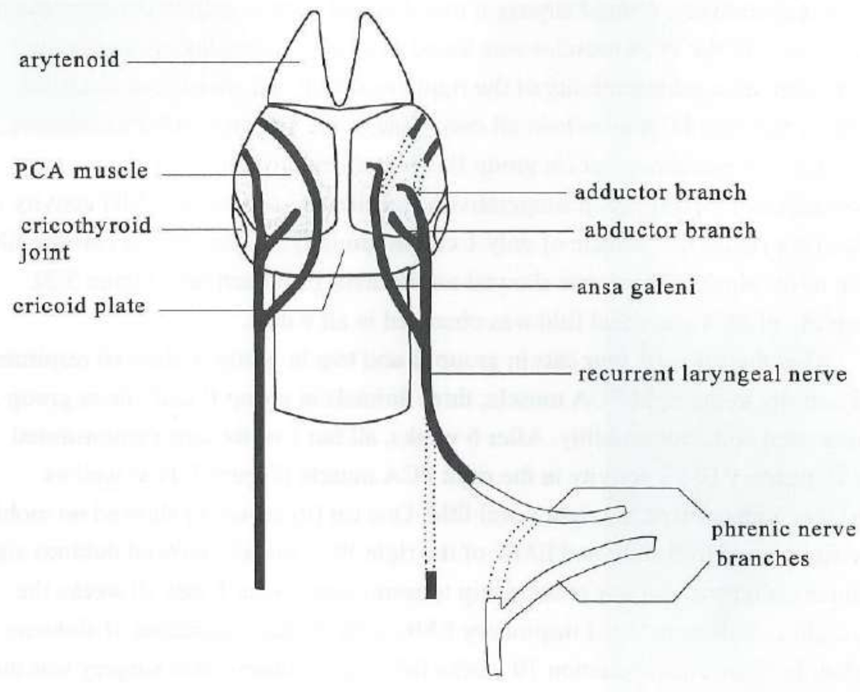


Figure 3.1 *Situation after selective laryngeal abductor reinnervation with a phrenic nerve graft on the right side. Anastomosis of the upper branch of the phrenic nerve with the recurrent laryngeal nerve. Proximal stumps of the adductor branch and ansa galeni are sutured into posterior crico-arytenoid (PCA) muscle.*

adduction of the right vocal fold, all these muscles were severed bilaterally before the final assessment. Furthermore, histological examination of the nerve anastomosis was performed to obtain histological proof of reinnervation and to quantify the amount of reinnervating axons. The whole complex of the transferred PN root, the nerve-anastomosis, and the right RLN was fixed in situ in the anesthetized animal using a cacodylate-gluteraldehyde solution and was then resected for histological examination. After postfixation in the same solution, the tissue was embedded in epon and 1-μm-thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5cm distally from the anastomosis, by computerized screening of a 500-μm-wide band across the widest nerve diameter. The axon count, axon diameter and thickness of the myelin sheath were estimated.

Results

Overall results

Preoperatively, normal laryngeal mobility and normal respiratory-dependant EMG activity of the PCA muscles was found in all cats. Immediate postoperative examination revealed immobility of the right vocal fold and absence of electrical activity in the right PCA muscle in all cats. Nine of the 10 cats could be evaluated (Table 3.1), the remaining cat (in group B) died of hemorrhage a few days postoperatively. Two weeks postoperatively, respiratory-dependant EMG activity was found in the right PCA muscle of only 1 cat (in group B). In the other 8 cats the EMG pattern of the right PCA muscle showed no coherent EMG activity (Figure 3.2). Immobility of the right vocal fold was observed in all 9 cats.

After 4 weeks, all four cats in group B and two in group A showed respiratory EMG activity in the right PCA muscle; three animals in group B and one in group A demonstrated abductor mobility. After 6 weeks, all but 1 of the cats demonstrated some inspiratory EMG activity in the right PCA muscle (Figure 3.3) as well as inspiratory abduction of the right vocal fold. One cat (in group A) showed no mobility of the right vocal fold at all and EMG of the right PCA muscle showed dubious signs of reinnervation without any relationship to respiration. After 8 and 10 weeks the same eight cats demonstrated inspiratory EMG activity and restoration of abductor function. In 6 cats, the abduction 10 weeks following reinnervation surgery was more outspoken on the operated right side than on the intact left side. In 1 cat, the abduction on the right side was slightly less than on the left, and in another it was equivalent to the abduction on the left.

Table 3.1 Overall results of right laryngeal abductor function in 9 cats *

	No. of Cats	
	EMG Activity (R PCA muscle)	**Abductor Mobility (Laryngoscopy)**
Time		
Preoperative	9	9
Postoperative, week		
0	0	0
2	1	0
4	6	4
6	8	8
8	8	8
10	8	8 (6R>L)
Hypoxia	8	8 (6R>L)
Electrical stimulation of PN	8	8

EMG indicates electromyography; PCA, posterior cricoarytenoid; PN, phrenic nerve; and R>L, ight abduction mobility greater than left. There was histological proof of reinnervation in 7 cats as ssessed 10 weeks postoperatively. In 1 cat (without restoration of abductor function), the nastomosis between the recurrent laryngeal nerve and the PN could not be identified owing to urgical failure, and in another cat the anastomosis could not be evaluated owing to unsuccessful xation. Electrical stimulation of the PN was done 10 weeks postoperatively.

In the situation of respiratory distress, the EMG activity of both left and right 'CA muscles increased in 8 cats, as did the abduction of the right and left vocal folds. gain, in 6 of these 8 cats the abduction was more pronounced on the right side. lectrical stimulation of the right PN resulted in abduction of the right vocal fold in 8 ats.

In the only cat without restoration of abductor function, the anastomosis etween the RLN and the PN could not be identified. This case is considered a urgical failure. In all other cats the anastomosis was identified. Histological proof of einnervation was obtained in 7 cats. One anastomosis (in group A) could not be valuated histologically due to unsuccessful fixation.

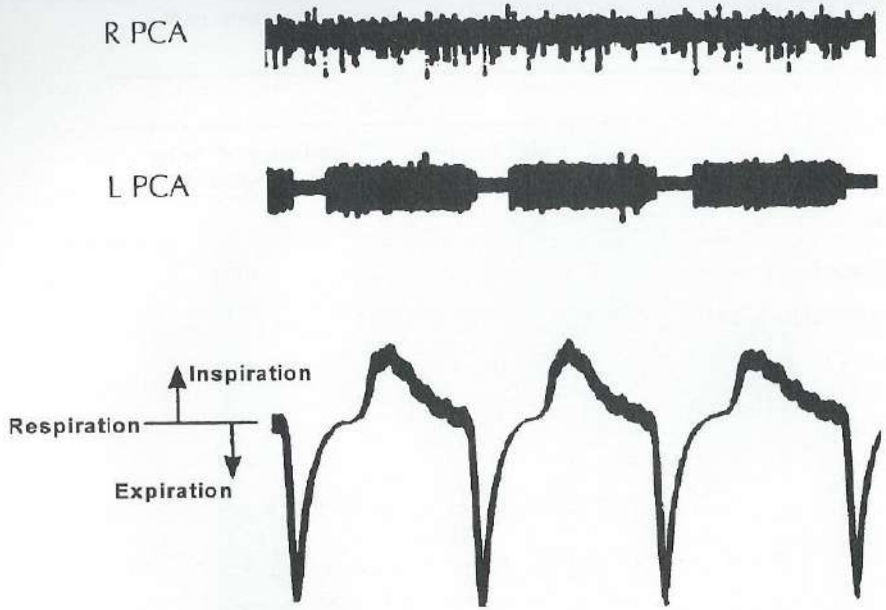


Figure 3.2 *Electromyography (0.5cm/sec) 2 weeks after selective laryngeal abductor reinnervation procedure on the right side. Note the normal inspiratory EMG in the left posterior cricoarytenoid (L PCA) muscle, no respiratory-dependant EMG in the right posterior cricoarytenoid (R PCA) muscle.*

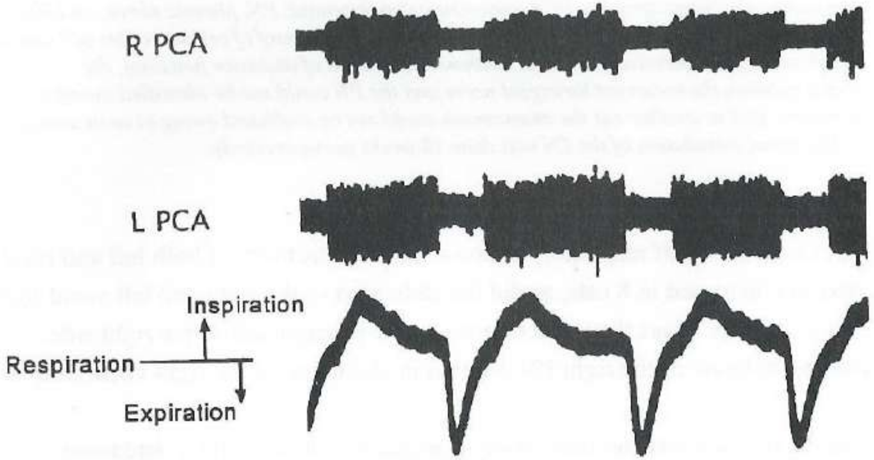
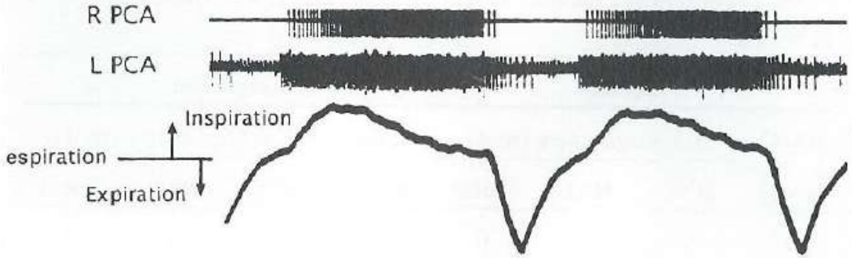


Figure 3.3 *Electromyography (0.5cm/sec) 6 weeks after selective laryngeal abductor reinnervation procedure on the right side. Note similar inspiratory EMG in both the right posterior crico-arytenoid (R PCA) muscle and the left posterior cricoarytenoid (L PCA) muscle.*

Specific EMG recordings and videolaryngoscopic observations

In the EMG registrations as well as the videolaryngoscopic evaluations a ypical PN activity pattern was observed in the right PCA muscle: the EMG activity Figure 3.4) and abduction on the right, started 30 to 40 milliseconds later than on the eft side. Furthermore, the right PCA muscle showed a slowly increasing EMG ıctivity at the onset of inspiration and an abrupt end of activity at the end of nspiration. The normal left PCA muscle almost immediately reached its maximal ıctivity at the onset of inspiration and slowly decreased towards the end. The resting EMG baseline activity, which was present during expiration in the normal left PCA nuscle, was absent in the reinnervated right PCA muscle.



igure 3.4 *Electromyography (2cm/s) 6 weeks after selective laryngeal abductor reinnervation rocedure on the right side. Note the slight delay in onset of the EMG activity in the right posterior ricoarytenoid (R PCA) muscle, the slowly increasing EMG activity in the R PCA muscle, and the bsence of EMG activity in the R PCA muscle during expiration when compared with normal ıspiratory EMG activity in the L PCA muscle.*

)rg 2766 versus Placebo

For comparison of Org 2766 with placebo, the EMG and videolaryngoscopic ecordings of 8 cats (4 in group A, 4 in group B) were evaluated. One cat in group B vas excluded because of death due to postoperative hemorrhage and 1 cat in group A vas excluded because it was considered a surgical failure. Furthermore, histological valuation was performed in 7 of these cats (3 in group A, 4 in group B); 1 cat in roup A was excluded because of unsuccessful fixation.

Solution A proved to be placebo and solution B contained Org 2766. Both MG and videolaryngoscopic recordings showed a tendency toward earlier return of activity of the PCA muscle in the Org 2766-treated group. After 4 weeks all of the 4 cats in group B demonstrated EMG activity, compared with only 2 of the 4 cats in group A; first signs of abduction were observed in 3 cats in group B and only in 1 in group A. Furthermore, return to normal or more than normal abduction seemed to be earlier in the cats in group B (Table 3.2).

Histological evaluation 0.5cm distal from the anastomosis showed no difference between groups A and B concerning myelin thickness and axon diameter. The mean axon density was, however, 26% higher in the Org 2766-treated group (6679 ± 1207[mean ±SEM] axons per square millimeter) than in the placebo-treated group (5301 ± 1186 axons per square millimeter). This difference in axon density is not statistically significant owing to the small number of cats.

Table 3.2 Org 2766 versus Placebo: Effect on right laryngeal abductor activity in 9 cats*

	Placebo				Org 2766			
	EMG	Laryngoscopy (n=4)			EMG	Laryngoscopy (n=4)		
Week	(n=4)	R<L	R≥L	Total	(n=4)	R<L	R≥L	Total
2	0	0	0	0	1	0	0	0
4	2	0	1	1	4	3	0	3
6	4	3	1	4	4	3	1	4
8	4	2	2	4	4	1	3	4
10	4	1	3	4	4	0	4	4

* *EMG indicates electromyography; R<L, right abduction activity less than left; and R≥L, right abduction mobility equal to or greater than left.*

Discussion

This study has demonstrated that unilateral selective laryngeal abductor reinnervation with a PN transfer is a highly effective procedure to restore laryngeal abductor function in cats. These results strongly support the findings of Baldiserra and coworkers (1986). Although the excellent restoration of abduction achieved in cats can not be simply translated to the human situation, we feel that the described technique holds promises for laryngeal reinnervation in humans as well. Some facts should be taken into consideration, however.

Firstly, the reinnervation surgery was performed immediately following the RLN section. Normally, in patients there is a considerable delay between the time of the nerve injury and the moment that therapeutical intervention is taken into consideration.

Secondly, this study involved only unilateral RLN lesions. It remains debatable whether patients with unilateral paralysis of the RLN should undergo reinnervation surgery. The main problem following unilateral RLN paralysis is usually dysphonia, which can successfully be treated with laryngeal framework surgery or slightly less successfully with Teflon or collagen injections. If reinnervation surgery is considered in unilateral RLN paralysis, it is only to improve the voice by increasing the tonus of the paralyzed vocal fold, without expectations of restoring mobility (Crumley et al. 1986). For this purpose a nonselective ansa cervicalis nerve transfer seems to be suitable (Crumley et al. 1991).

Thirdly, there is the trade-off between the morbidity of sacrificing a PN nerve and the morbidity of a bilateral RLN nerve palsy. The morbidity of unilateral PN palsy is considered to be minor and transection of one PN is generally well tolerated without negative pulmonary side-effects (Fackler et al. 1967, Robotham 1979, Easton et al. 1983, Kelly 1950). This may be because the diaphragm is innervated not only by the PN proper, but also by accessory PNs and thoracic branches (Kelly 1950). Bilateral transection of the PN, however, is associated with a higher morbidity. Therefore, selective abductor laryngeal reinnervation with a PN transfer can only be performed unilaterally, or bilaterally if only one PN is used, as has been described by Baldiserra and coworkers (1989). Considering the remarkably good restoration of laryngeal abduction in this study, a unilateral PN reinnervation might even result in a sufficient airway in bilateral RLN palsy.

Ideally, not only the respiratory abductor function, but also the adductor phonatory function, should be restored. This might be achieved by an additional ansa cervicalis transfer to the adductor branch of the RLN. However, the activity patterns of the ansa cervicalis nerve branches are not specific for phonation. The ansa cervicalis shows a rather weak activity throughout the respiratory cycle, with a minor increase during inspiration. Improvement of tonus of the adductor muscles due to ansa cervicalis nerve transfer, more than actual adduction, is held responsible for the voice improvement observed following this procedure (Crumley et al. 1986, Crumley 1991). Such an improvement of tonus might be important in combined selective abductor and adductor laryngeal reinnervation to counterbalance the activity of the reinnervated abductor muscles during inspiration, thus preventing an otherwise flaccid vocal fold to be sucked caudo-medially by the inspiratory airstream. Furthermore, this study demonstrated that the resting activity of the PN, reinnervated laryngeal abductor during expiration is markedly less than the resting activity of the normal laryngeal abductor. In this situation of extreme antagonistic relaxation, the ansa cervicalis activity in the adductor muscles might be able to elicit a laryngeal adduction during the expiratory phase for phonatory purposes.

In addition, reinnervation of the adductor muscles with the ansa cervicalis will prevent axonal escape of PN axon sprouts from the PCA muscle to the adductor muscles. Although it was not observed during the ten week follow-up in this study, such an axonal escape is likely to occur towards denervated muscles. Innervated muscles, however, are not susceptible to ingrowing "foreign" axons. Axonal escape of PN axons into the laryngeal adductors would of course result in laryngeal synkinesis.

The results obtained following treatment with Org 2766 are not conclusive, owing to the small number of cats involved. However, the tendency towards facilitation of axon sprouting by Org 2766 (26% more axons) in the present study is of comparable magnitude as reported for facilitation of regeneration of crushed sciatic nerve in rats (32% more axons) (Tonnaer et al. 1992).

The tendency toward a more rapid innervation and a higher number of reinnervating axons is a very interesting observation. Especially in muscles with small motor units, like the laryngeal muscles, the number of reinnervating axons might be of great importance to restoration of function. More axons enable the formation of smaller motor units and consequently a more finely graded control of muscle contraction. The results of this pilot study justify further research, both concerning the concept of selective laryngeal reinnervation and the use of Org 2766 to stimulate axon sprouting.

Chapter 4

Selective Laryngeal Reinnervation with Separate Phrenic and Ansa Cervicalis Nerve Transfers

Julie T van Lith-Bijl (MD), Robin J Stolk,
Jeroen ADM Tonnaer (PhD), Cees Groenhout (MD),
Pierre NM Konings (PhD), Hans F Mahieu (MD,PhD).

Archives of Otolaryngology, Head & Neck Surgery 1997;123:406-411

Chapter 4

Selective Laryngeal Reinnervation with Separate Phrenic and Ansa Cervicalis Nerve Transfers

Julie T van Lith-Bijl (MD), Robin J Stolk,
Jeroen ADM Tonnaer (PhD), Cees Groenhout (MD),
Pierre NM Konings (PhD), Hans F Mahieu (MD,PhD).

Archives of Otolaryngology, Head & Neck Surgery 1997;123:406-411

reinnervation of both adductor and abductor muscles and synkinesis (Lahey et al. 1938, Ballance 1924, Barnes et al. 1927, Crumley et al. 1986); if selective reinnervation was attempted, donor nerves without specific respiratory or phonatory activity patterns were often used, resulting in reinnervation with inappropriate activity for laryngeal function (Tucker 1975, 1978); and subclinical reinnervation (Elsberg 1917, Edds 1949, 1953, Gwynn et al. 1966) that usually occurs in cases of long-standing laryngeal palsy, without clinical signs of reinnervation. Since subclinically innervated muscles are not susceptible to reinnervation, surgical reinnervation will fail if the subclinical innervation pathway is not severed. For example, a frequently applied laryngeal reinnervation procedure, Tucker's nerve muscle pedicle reinnervation of the posterior cricoarytenoid (PCA) muscle (Tucker 1975, 1978) has the disadvantages of the last 2 causes of failure to restore laryngeal mobility. This technique uses a branch of the ansa cervicalis, the inspiratory activity of which is too weak in quiet breathing to activate the abductor muscles. Only in respiratory distress conditions the branches of the ansa cervicalis show a significant inspiratory activity. Furthermore, the original innervation path (ie the RLN) remains intact in Tucker's procedure, which is likely to contain subclinically reinnervating axons, keeping the muscles in a condition resistant to reinnervation.

Reinnervation of the adductor musculature in patients with unilateral laryngeal paralysis, using a non-selective procedure anastomosing the ansa cervicalis to the main trunk of the RLN, has resulted in voice improvement (Crumley et al. 1986, Crumley 1991). This improvement must be attributed to the restoration of muscle tonus rather than actual adduction. Restoration of functional mobility can be obtained only by using separate selective surgical reinnervation with donor nerves with appropriate activity patterns for the abductor and adductor muscles. This procedure can prevent synkinesis and result in adequate muscle activity.

In our earlier study (Chapter 3) and the work reported by Baldiserra and coworkers (1986) a selective laryngeal abductor reinnervation with a phrenic nerve (PN) transfer has proved an effective procedure to restore adequate laryngeal abductor function during inspiration in cats. The inspiratory abductor, as well as the adductor function, ideally should be restored. Laryngeal adduction is a key element in airway protection, phonation, and coughing and the regulation of airway resistance.

Unlike the inspiratory activity of the PN, no adequate donor nerve with appropriate activity is available for laryngeal adduction during phonation, swallowing, effort closure, vomiting and coughing.

The ansa cervicalis branches, which are often suggested for adductor reinnervation, display a very weak inspiratory activity pattern that increases during the respiratory distress condition. There is a slight increase in activity during swallowing, coughing and straining (Ellenbogen et al. 1981, Fink 1975). There is also a difference in the histochemical and contractile properties of the adductor and strap muscles and their nerve supply. Muscle fibers can be divided into specific types based on their histochemical and contractile properties (Fata et al. 1987). The following are 3 basic groups: type 1, slow contractile period and anaerobic metabolism; type 2B, fast contractile period and anaerobic metabolism; type 2A, intermediate contractile period and both aerobic and anaerobic metabolism. Muscles with low tension during a sustained period will have a high concentration of type 1 muscle fibers. A high concentration of type 2B fibers is seen in muscles that contract rapidly generating high tension during a short time. The thyroarytenoid muscle (part of which is the vocalis muscle of the vocal fold) contracts quickly (14 milliseconds) and has less than 36% of type 1 muscle fibers. The strap muscles, however, have a higher percentage of type 1 muscle fibers (almost 66%) and a contraction time of 50 milliseconds (Hast 1968). Therefore, although the branches of the ansa cervicalis are not ideal as donor nerves for laryngeal adductor reinnervation, they are a realistic alternative. Adductor reinnervation with the ansa cervicalis will at least result in a resting muscle tonus.

Furthermore, our previous study (Chapter 3) demonstrated that the resting activity of the laryngeal abductor, reinnervated using the PN, during expiration, is less than that of the normal abductor activity of the RLN. In extreme antagonistic relaxation, the activity of the ansa cervicalis activity in the adductor muscles might be able to elicit laryngeal adduction during the expiratory phase for phonation.

To test the feasibility of selective adductor and abductor reinnervation, we combined the selective reinnervation of the laryngeal abductor using the PN with a selective laryngeal adductor reinnervation using an ansa cervicalis sternohyoid branch to restore both laryngeal abductor and adductor function.

Materials and methods

Surgical method

Ten female cats (age, 6 months; weight range, 2200g to 2800g) were anesthetized with ketamine hydrochloride 20mg/kg intramuscularly and xylazine hydrochloride 0.5mg/kg subcutaneously, allowing spontaneous respiration. Using a midline incision in the neck, the larynx, the proximal trachea and the right RLN were exposed. The right ansa cervicalis was then identified and followed to its sternohyoid branch. The sternohyoid branch was severed near the sternohyoid muscle. The proximal stump was then transferred and anastomosed to the adductor branch of the RLN. The larynx was then rotated 90° along its longitudinal axis. The inferior pharyngeal constrictor muscle was sectioned at the insertion on the inferior margin of the right thyroid lamina. A small, inferior, posterior part of the thyroid lamina was resected to expose the adductor and abductor nerve branches of the RLN. Preserving the abductor branch and severing the adductor branch, the proximal adductor stump was buried in the PCA muscle and fixed with a 10-0 microsuture (Ethilon, Ethicon Inc, Somerville NJ) and fibrin glue (Tissucol, Austrian Institute for Haemoderivates, Vienna, Austria). The distal adductor stump was anastomosed to the proximal stump of the sternohyoid branch of the ansa cervicalis with a 10-0 microsuture and fibrin glue. To eliminate possible influence of the ansa galeni or Galen's anastomosis, although unlikely because of its allegedly sensory nature (Diamond et al. 1992), it was also severed and its proximal stump buried and fixed in the right PCA muscle with fibrin glue.

The 2 roots of the right PN were identified. Unlike humans, PN in the cat has two roots. The uppermost PN root was resected just before the intersection with the other root. The right RLN was severed 2.0cm caudal to the cricothyroid joint and the distal stump of the RLN was anastomosed to the proximal stump of the PN using a 10-0 microsuture and fibrin glue. Thus all reinnervating PN axons were directed towards the PCA muscle, even those that followed the path of the adductor branch or the ansa galeni (Figure 4.1).

Assessment of laryngeal function

To assess laryngeal abductor and adductor function, videolaryngoscopy and electromyography (EMG) were performed, using the same anesthesia that was used for the surgical procedure. Laryngeal function was assessed before and immediately after reconstruction; thereafter, it was assessed weekly during a 10-week follow-up period. The time at which the first sign of mobility and EMG reinnervation activity was seen was recorded.

After 10 weeks, laryngeal abductor function was evaluated during quiet espiration and respiratory distress. The respiratory distress condition was achieved by performing a tracheotomy and occluding the tracheotomy tube (type Shiley 00 CFS; Mallinkrodt Medical, Irvine, CA) for 1 minute. To eliminate any influence of the cricothyroid muscles or extrinsic laryngeal musculature, these muscles were severed

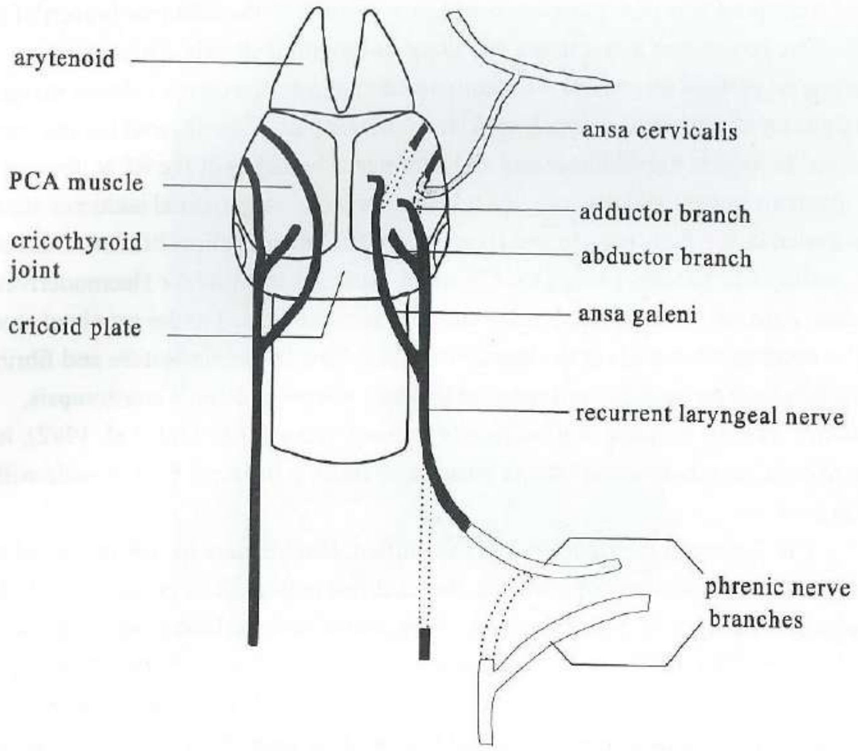


Figure 4.1 *Anastomosis of the sternohyoid branch of the ansa cervicalis with the adductor branch of the right recurrent laryngeal nerve; anastomosis of the upper branch of the phrenic nerve with the recurrent laryngeal nerve. Proximal stumps of the adductor branch and ansa galeni are sutured into the posterior cricoarytenoid muscle.*

bilaterally prior to the final assessment. The superior laryngeal nerve (SLN) was preserved. Reflex adductor movement was evaluated by recording mobility and EMG activity on tactile stimulation of the right-sided supraglottic mucosa. The RLN-PN and ansa-adductor anastomoses were then identified and electrically stimulated (1mAmp, 50 Hz) proximal to the anastomosis, the laryngeal response was observed by videolaryngoscopy and EMG.

Videolaryngoscopic evaluation

The mobility of the reinnervated right hemilarynx was compared with the normal left hemilarynx. Abduction was scored according to the following: *good*, if abduction on inspiration occurred almost synchronous with and equal in degree of

abduction to the abduction of the left side; *adequate*, if abduction on inspiration was more than half but slightly less than the abduction of the left side; *limited*, if abduction on inspiration was less than half or half of the abduction of the left side; or *poor*, if no effective or slight abduction on inspiration was observed. Adduction was scored according to the following: *normal*, if right hemilarynx adduction was equal to that of the left hemilarynx; *decreased*, adduction of the right side was less than the left side; *immobile*, if the right hemilarynx was immobile; and *paradoxical*, if abduction of the right side occurred during adduction of the right side.

EMG evaluation

Electromyography was performed by means of transorally introduced, hooked wire electrodes in the left and right posterior cricoarytenoid (PCA) muscle, and in the left and right vocalis muscles (medial part of the thyroarytenoid muscle). An EMG type MS6 (Medelec, Old Walking, England) was used. Respiratory monitoring was performed simultaneously, using a custom-made impedance plethysmograph, that registered chest movement.

Histology

To obtain histological proof of the reinnervation, nerve anastomoses were fixed in situ in the anesthetized animal using a cacodylate-gluteraldehyde solution and resected for histological examination. After postfixation in the same solution, the tissue was embedded in epoxy resin and 1-µm-thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5cm distal to the anastomosis, by computerized screening of a 500-µm-wide band across the widest nerve diameter. The number of axons per square millimeter and the axon diameters were estimated.

Results

Preoperative assessment

Before the operation, all cats showed symmetrical spontaneous laryngeal abduction on inspiration and symmetrical adduction on tactile stimulation of the right-sided supraglottic mucosa (reflex adduction). EMG activity patterns were normal in all muscles recorded. The PCA muscles showed an inspiratory activity pattern. In the vocalis muscles, a quiet baseline activity was recorded throughout the respiratory cycle. On reflex adduction a burst of activity was recorded in both vocalis muscles.

In 1 cat iatrogenic damage to the right RLN occurred before performance of the PN-RLN anastomosis, precluding successful anastomosis. This case was not evaluated. The other 9 cats could be evaluated.

Table 4.1 Laryngeal abduction mobility observed with videolaryngoscopy 10 weeks after selective laryngeal reinnervation

Abduction of Right Hemilarynx	No. of cats	
	Quiet Respiration	Respiratory Distress
Good	6	5
Adequate	2	2
Limited	0	2
Poor	1	0

Table 4.2 Laryngeal reflex adduction mobility observed with videolaryngoscopy 10 weeks after selective laryngeal reinnervation

Reflex Adduction of Right Hemilarynx	No. of cats
Normal	0
Decreased	2
Immobile	7
Paradoxical	0

Intermediate assessment

Immediately after the operation the right hemilarynx was immobile in all 9 cats. Denervation of the right RLN was confirmed by EMG inactivity in the right vocalis muscle and the right PCA muscle.

A first sign of spontaneous mobility of the right vocal fold, comprising a slight trembling movement on inspiration was seen with videolaryngoscopy an average of 4.8 weeks after the operation in 8 cats (range, 3-6 weeks). In 1 cat the right vocal fold was still immobile 10 weeks after the operation, although the EMG patterns showed appropriate reinnervation. In 9 cats, the results of EMG indicated reinnervation of the right vocalis muscle an average of 4.8 weeks after the operation (range, 4-6 weeks) and of the right PCA an average of 4.7 weeks after the operation (range, 3-7 weeks).

These recovery periods did not differ statistically (Mann-Whitney *U* test).

Final assessment

Videolaryngoscopic findings are shown in Table 4.1 and Table 4.2. The cat with poor abduction during quiet respiration showed a slight abduction during respiratory distress. The results of EMG, however, showed appropriate reinnervation. Palpation of the cricoarytenoid joint disclosed a reduced mobility of the joint, possibly due to fibrosis caused by weekly repeated manipulation with EMG electrodes or during the initial reinnervation surgery. In the other cats videolaryngoscopic evaluation during quiet respiration showed smooth abduction on inspiration, which occurred slightly later on the right side than on the left. During respiratory distress, videolaryngoscopy typically showed bulging of the vocal fold, together with a decrease of right-sided abduction. The abduction movement on the right side was slower and jerkier than on the left side. The jerky movement persisted during sustained abduction. The results of EMG of the reinnervated right PCA muscles showed a typical PN activity pattern in 9 cats. The EMG activity and abduction on the reinnervated right, started 30 to 40 milliseconds later than on the left side. Furthermore, EMG activity slowly increased at the onset of inspiration; the activity decreased slightly and abruptly stopped at the end of inspiration. The normal left PCA muscle almost immediately reached its peak activity at the onset of inspiration and

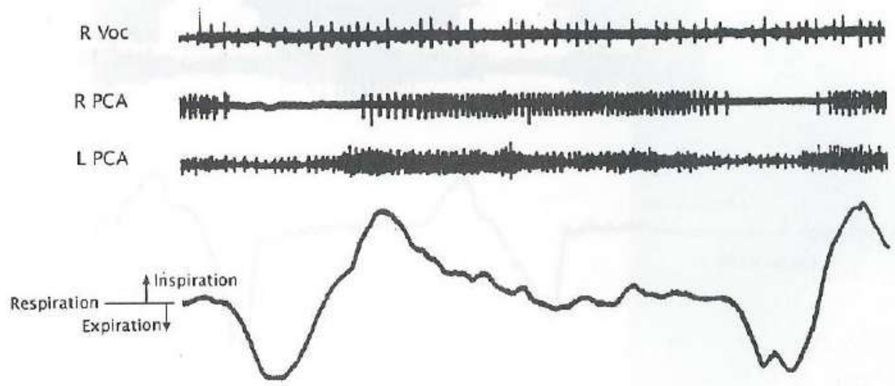


Figure 4.2 *Top, Electromyogram (2cm/s) of quiet respiration, 10 weeks after selective reinnervation procedure on the right side. Minor inspiratory activity in the right vocalis muscle (R Voc) is typical for the ansa cervicalis during quiet respiration. The inspiratory activity is typical for the phrenic nerve in right posterior cricoarytenoid (R PCA) muscle almost synchronous with the activity in the left posterior cricoarytenoid (L PCA) muscle. Bottom, The respiratory cycle.*

slowly decreased at the end of inspiration. The resting, baseline EMG activity, which was present during expiration in the normal left PCA muscle, was absent in the reinnervated right PCA muscle in all cats.

The EMG activity in the reinnervated right vocalis muscle, had a weak inspiratory pattern which started later than that in the left or right PCA muscles and only consisted of a single motor unit potential in 7 cats. In 2 cats no activity was seen during quiet breathing, whereas there was evidence of a firm inspiratory pattern, recruiting multiple motor units, during respiratory distress. In the other 7 cats, the EMG activity increased in the right vocalis muscle during the respiratory distress. This activity pattern is typical for the ansa cervicalis.

Examples of the EMG activity of the right vocalis muscle and right and left PCA muscles during quiet respiration and respiratory distress are shown in Figure 4.2 and Figure 4.3, respectively.

No EMG activity was recorded during reflex adduction in the right vocalis muscle or in the right and left PCA muscles. A normal response activity was recorded in the left vocalis muscle.

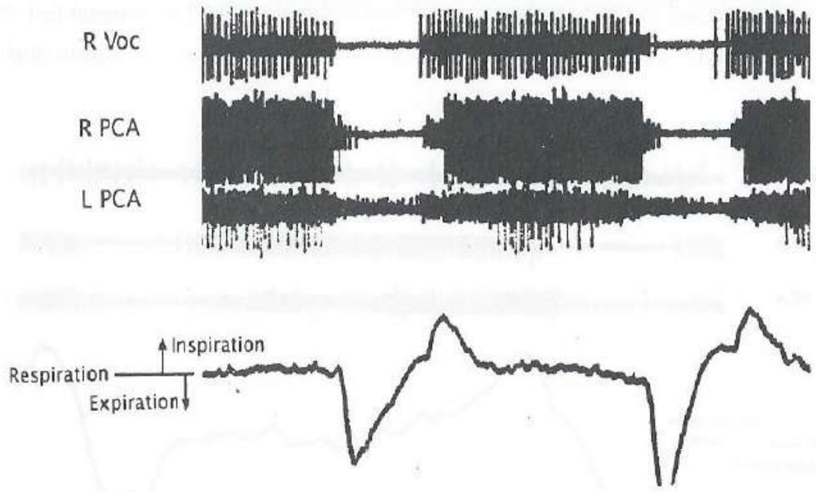


Figure 4.3 *Top, Electromyogram (2cm/s) of respiratory distress 10 weeks after the selective reinnervation procedure on the right side. Note the inspiratory activity in the right cricoarytenoid (R PCA) muscle, which is typical for the phrenic nerve, and the marked simultaneous inspiratory activity in the right vocalis muscle (R Voc), which is typical for the ansa cervicalis during respiratory distress. This synkinetic activity only slightly compromised the abductor movement as observed laryngoscopically (not shown). Bottom, The respiratory cycle.*

Electrical stimulation of the PN showed abduction of the right vocal fold in 9 cats, and an EMG activity response was recorded simultaneously in the right PCA muscle. Electrical stimulation of the right ansa cervicalis sternohyoid branch showed adduction of the vocal cord in 9 cats. In the right vocalis muscle, an EMG response activity was recorded simultaneously.

Histological analysis

The PN-RLN anastomosis could be histologically examined, but an examination was not feasible for the ansa-adductor nerve anastomosis due to the small caliber and extensive fibrosis near the anastomosis which precluded proper preparation. Evaluation of the distal part of the right RLN was performed in 8 of the 9 cats, and histological proof of reinnervation was obtained in these specimens. The remaining case could not be evaluated because of unsuccessful fixation. The median axon count was 3097 axons per square millimeter, with a median axon diameter of 1.97μm. (In one of our previous studies (Chapter 2) involving cats of the same breed, sex and weight range as were used in this study the median axon count in the unaffected RLN was 3144 axons per square millimeter and the median axon diameter was 4.68μm).

Discussion

To our knowledge, few attempts have been made in animal experiments to simultaneously combine a selective reinnervation of the laryngeal abductor and adductor muscles using the phrenic nerve and ansa cervicalis, respectively. Crumley (1984) reported performing such a procedure in 2 dogs. In both dogs, reinnervation was achieved, and the results were comparable with those of our present study. Crumley's method differed in that respect that he used a nerve graft to connect the phrenic nerve to the intralaryngeal abductor branch and the sternothyroid branch of the ansa cervicalis. Marie and coworkers (1989) used a similar procedure in 7 dogs: direct implantation of a phrenic nerve stump in the PCA in combination with the ansa cervicalis sternothyroid branch to the adductor branch. Eight months later functional reinnervation of both adductor and abductor muscles was obtained in only one dog with good abduction. Adduction was restored in 5 dogs. The main reason for failure of restoration of abductor function besides avulsion in 1 dog and infection in 1 dog appeared to be synkinesis resulting from "axonal escape".

Our present study shows that a good laryngeal function can be achieved in cat during quiet respiration when combining abductor reinnervation with a PN and adductor reinnervation using the ansa cervicalis branch. A minor compromise of the maximal abduction is found during respiratory distress due to simultaneously increased inspiratory activity in the PN and ansa cervicalis under this condition, as compared to the abduction during quiet respiration. This is in contrast to our previous study (Chapter 3) in which abductor and no adductor reinnervation was performed, resulting in an increased abduction during respiratory distress. The results of EMG of the vocalis muscles and electrical stimulation of the ansa cervicalis indicated that the adductors were indeed reinnervated, and, thus, a muscle tonus had been achieved in the right vocalis muscle. This might be advantageous in phonation as in unilateral recurrent nerve paralysis. Achieving only muscle tonus in the vocal fold, without active adduction, has been shown to improve voice quality (Crumley et al. 1986, Crumley 1991). Furthermore, this muscle tonus may be advantageous during respiration, especially during quiet inspiration and inspiration during respiratory distress, by preventing an otherwise flaccid vocal fold to be sucked caudo-medially by the inspiratory airstream.

In theory, reinnervating the adductor muscles with the ansa cervicalis might prevent axonal escape of PN axon sprouts to the adductor muscles and might thus prevent laryngeal synkinesis, since (re)innervated muscles, as opposed to denervated muscles, are insusceptible to ingrowing foreign axons. However, we did not observe PN activity in the adductor muscles in our earlier study (Chapter 3) in which only the abductor reinnervation was performed.

Tactile stimulation of the right supraglottic mucosa resulted in an adductor response on the left side but not on the right side as expected, since the ansa cervicalis is not involved in the reflex arc. The protective reflex arc is triggered by stimulation of tactile receptors in the glottic and supraglottic mucosa. The stimulus passes along the sensory fibers of the internal branch of the superior laryngeal nerve (SLN) via the nodose ganglion to the nucleus solitarius, which is the nucleus of the SLN in the brain stem. There is a polysynaptic connection to the nucleus ambiguus and the motor fibers of the RLN, evoking contraction of the laryngeal adductor muscles and inhibition of the phasic inspiratory action potentials in the PCA muscles (Kirchner 1991, Isogai et al. 1991, Sasaki et al. 1976). In the cat, there is a bilateral glottic closure response due to crossing axons, whereas this reflex is strictly unilateral in humans (Sasaki et al. 1976). Therefore, the cats in our study still had a partly intact glottic closure reflex, in humans, tactile stimulation of the right-sided supraglottic mucosa following a similar reinnervation procedure would result in no adduction.

In this study the sternohyoid branch of the ansa cervicalis was chosen for the

ıdductor reinnervation because it is easy to locate in the cat. Since the concept of separate selective adductor and abductor reinnervation has proved feasible, it might be better, in theory, to use the thyrohyoid branch of the ansa cervicalis in future research models, as this branch has a slightly more pronounced expiratory and phonatory activity than the sternohyoid branch (Ellenbogen et al. 1981, Fata et al. 1987, Hast 1968, Diamond et al. 1992).

Alternative transfer of the SLN which is known to be active during phonation and coughing has been considered by Crumley (1984) for reinnervation. However, the cricothyroid muscle normally functions as an accessory adductor and its adductory activity is especially of importance in larynges with RLN paralysis. By transferring the SLN to the adductor branch one would be as Crumley describes, "robbing Peter to pay Paul", which would hardly be advantageous.

Since phonation could not be evaluated in this study and effective reflex glottic closure was not achieved, it remains to be proven that reinnervation of the adductor musculature is a meaningful addition to selective abductor reinnervation alone. The theoretical advantages of adductor muscle tonus and the favorable experience in reinnervation with ansa cervicalis branches in unilateral laryngeal paralysis in humans to improve phonation, together with the minor additional morbidity of sacrificing an ansa cervicalis nerve branch, in our opinion, justifies further development of selective abductor and adductor reinnervation and its eventual use in humans. The results obtained with selective reinnervation are much better than those which can be achieved by direct RLN reanastomosis which results in gross laryngeal function impairment due to synkinesis (Chapter 2).

Chapter 5

Laryngeal Abductor Reinnervation with a Phrenic Nerve Transfer after a 9-Month Delay

Julie T van Lith-Bijl (MD), Robin J Stolk (MD),
Jeroen ADM Tonnaer (PhD), Cees Groenhout (MD),
Pierre NM Konings (PhD), Hans F Mahieu (MD,PhD).

Archives of Otolaryngology, Head & Neck Surgery, 1998;124:393-398

Abstract

Objective To test the feasibility of delayed selective abductor reinnervation following transection of the recurrent laryngeal nerve (RLN). Successful restoration of laryngeal abductor function, using the phrenic nerve has been described in the cat model, in the acute phase. However, in clinical practice there is usually a considerable delay between injury to the RLN and presentation for treatment. Delayed reinnervation therefore would be more suitable in clinical practice.

Materials and methods In 12 cats, the right RLN was severed. Nine months later, the phrenic nerve was anastomosed to the distal RLN stump with all its branches directed toward the posterior cricoarytenoid muscle. For 10 weeks after the reconstruction, electromyography and videolaryngoscopy were performed weekly. Finally, histological analysis of the RLN was performed.

Results Evaluation was possible in 11 cats. Reinnervation of the right posterior cricoarytenoid muscle with the phrenic nerve occurred in 10 cats following nerve anastomosis, but results of videolaryngoscopy showed adequate to good abduction in only 4 cats. The main limiting factor was reduced mobility of the cricoarytenoid joint. Evidence of spontaneous subclinical reinnervation after the delay was observed in 7 cats but apparently did not impede the surgical reinnervation.

Conclusion Delayed selective laryngeal abductor reinnervation was feasible, but function recovery was less successful than if performed immediately. Future investigations should concentrate on early determinants of spontaneous restoration of function to allow early selection of patients who are eligible for reinnervation surgery.

Introduction

The diagnosis of RLN or vagus nerve paralysis often is made after a considerable delay, particularly in cases of unilateral paralysis, when the symptoms of dysphonia and sometimes aspiration may have been present for weeks or even months before a laryngological consultation is sought. In the less common case of bilateral (RLN or vagus nerve) paralysis, progressive stridor and dyspnea often lead to an earlier diagnosis. Unless respiratory insufficiency warrants urgent airway management, attention is first directed toward the cause, extent and site of the nerve injury. In addition, before any form of permanent and mostly irreversible surgical treatment is performed, spontaneous recovery or compensation (in case of unilateral paralysis) is awaited for some months. Therefore, a considerable delay between the onset of nerve injury and the timing of surgical intervention generally elapses.

In the case of persistent unilateral paralysis where dysphonia predominates, vocal fold augmentation (Ford 1991) or medialization laryngeal framework surgery (Isshiki et al. 1974, Mahieu et al. 1996) are the most suitable procedures to correct the incomplete glottis closure. For permanent bilateral paralysis, the present treatment options consist of vocal fold lateralization (Geterud et al. 1990), arytenoidectomy (Ossoff 1984), posterior transverse cordotomy (Dennis et al. 1989) or permanent tracheostomy. With the exception of permanent tracheostomy, all of these procedures are aimed at enlarging the airway at the glottic level at the inevitable cost of voice quality and an increased risk of aspiration.

Experimental work has therefore been directed towards reinnervation surgery (Chapter 3 & 4, Crumley 1982, Rice 1982) as well as laryngeal pacing procedures (Broniatowski 1985, Zealear 1994). Both methods aim to improve laryngeal function by restoring laryngeal mobility with opening of the airway during inspiration without sacrificing voice quality or increasing the risk of aspiration. Our previous studies (Chapter 3 & 4) have concentrated on reinnervation surgery as a potential technique to restore laryngeal function and have achieved excellent results using surgical reinnervation techniques immediately following nerve injury in an animal model.

In treatment of long-standing paralysis, many factors may adversely influence successful surgical reinnervation and laryngeal function restoration. These include denervation atrophy, spontaneous subclinical reinnervation, inappropriate reinnervation and, fixation of the cricoarytenoid joint. If extensive denervation atrophy has occurred, this may preclude successful reinnervation because of irreversible muscle fibrosis and degeneration of muscle endplates (Gutmann et al. 1949, Saito et al. 1969). Usually, however, some spontaneous reinnervation takes place due to axonal regeneration across the injury site or neurotization from surrounding musculature. The process of atrophy is then halted and reversed. Such reinnervation, generally will not provide laryngeal mobility, a condition known as subclinical (re)innervation (Elsberg 1917, Edds 1949). Alternatively, should the reinnervation be sufficient to produce adequate muscular contraction, the regenerated axons may connect to the wrong target muscle, causing synkinesis and thus impairment of function (Crumley et al. 1982, Chapter 2). This condition is referred to as inappropriate reinnervation. In either reinnervated condition the muscle is no longer susceptible to reinnervation or neurotization from other sources, thus precluding successful reinnervation surgery. These conditions can be diagnosed using laryngeal electromyography (EMG) which typically demonstrates EMG activity in the absence of laryngeal mobility. Subclinical reinnervation and inappropriate reinnervation, rather than true persistent denervation, seem to be the rule some time after serious nerve injury. Furthermore, in long-standing laryngeal immobility, the cricoarytenoid joints may become fixed. This phenomenon appears to be rare in humans, (Elies et al. 1983) but extensive fibrous ankylosis has been observed in rabbits after experimental section of the RLN (Langnickel 1973). This will of course impede restoration of laryngeal mobility despite successful reinnervation.

Considering these factors, time appears to be of paramount importance when contemplating laryngeal reinnervation surgery. Separate selective reinnervation of the adductor and abductor muscle groups can prevent inappropriate laryngeal reinnervation by misdirection of regenerating axons. This reinnervation technique has proved very effective in cats when performed in the acute stage, ie, immediately after denervation by transection of the RLN (Chapter 3&4). Little experimental data are available regarding laryngeal reinnervation surgery after a delay (Neal et al. 1981, Morledge et al. 1973, Lyons et al. 1974).

We performed this study to test the feasibility of selective abductor reinnervation of the posterior cricoarytenoid muscle using a phrenic nerve (PN) transfer after a delay of 9 months after RLN transection.

Materials and methods

Surgical method

Twelve female cats (age, 6 months; weight range, 2200-2800 g) were anesthetized with ketamine chloride (20mg/kg given intramuscularly) and xylazine hydrochloride (0.5mg/kg given subcutaneously), allowing spontaneous respiration. A midline incision of the neck was performed. Pretracheal muscles were separated in the midline, and the upper part of the trachea was exposed. The right RLN was then identified. At a distance of 2.5cm from the cricothyroid joint, the RLN was transected. The proximal RLN was then resected as far caudally as possible, usually a length of 4 to 5cm. The distal stump was then fixed in a small piece of plastic tubing to facilitate reidentification.

After 9 months, the neck was again explored using an extended midline incision. The distal RLN stump was identified and retransected slightly more distal to the initial transection, 2.0cm from the cricothyroid joint. The larynx was rotated 90° around its longitudinal axis. The inferior pharyngeal constrictor muscle was partly sectioned at its insertion on the lower posterior margin of the right thyroid lamina. A small inferior posterior part of the thyroid lamina was resected to expose the adductor and abductor RLN branches. The abductor branch was preserved, the adductor branch

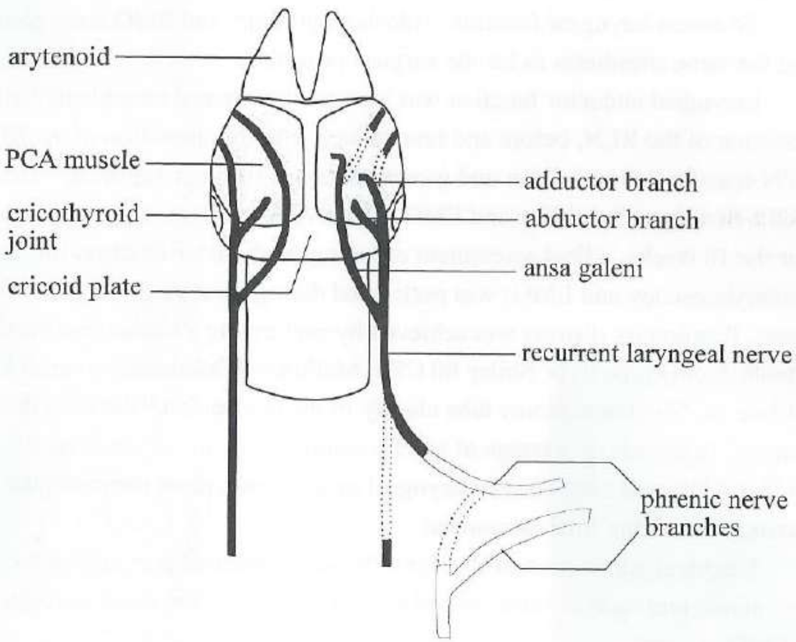


Figure 5.1 *Diagram of selective abductor reinnervation procedure anastomosing the phrenic nerve (PN) to the main stem of the recurrent nerve. To direct all regenerating axons from the PN to the posterior cricoarytenoid (PCA) muscle, the adductor branch and the ansa galeni were also cut and the proximal stumps were buried in the PCA muscle.*

/as severed and the proximal adductor stump was buried in the PCA muscle and fixed /ith a 10-0 microsuture (Ethilon, Ethicon Inc, Somerville NJ) and fibrin glue Tissucol, Austrian Institute for Haemoderivates, Vienna, Austria). To exclude any ıfluence of the ansa galeni, although unlikely because of its allegedly sensory nature Diamond et al. 1992) it too was severed, and its proximal stump was buried in the CA muscle. The two roots of the right PN were identified. In contrast to the situation ı humans, the PN in the cat has 2 clearly distinguishable roots originating from C-5 ıd C-6, respectively. The PN root from C-5 was transected just before it joined the ther root. The distal RLN stump was anastomosed to the C-5 PN stump using a 10-0 ıicrosuture and fibrin glue. Thus, all reinnervating PN axons were directed toward ıe PCA muscle (Figure 5.1), even those that followed the path of the adductor branch r the ansa galeni.

Laryngeal abductor function assessment

To assess laryngeal function, videolaryngoscopy and EMG were performed, using the same anesthesia as for the surgical procedure.

Laryngeal abductor function was assessed before and immediately after initial transection of the RLN, before and immediately after retransection of the RLN, before the PN-transfer 9 months later and weekly during a 10-week follow up. The time at which a first sign of mobility and EMG reinnervation activity were seen was recorded. After the 10 weeks, a final assessment of laryngeal abductor function (using videolaryngoscopy and EMG) was performed during quiet respiration and respiratory distress. Respiratory distress was achieved by performing a tracheotomy and occluding the tracheotomy tube (type Shiley 00 CFS; Mallinkrodt Medical, Irvine, CA, USA.) for 1 minute. The tracheotomy tube closely fit the trachea in all cases so that on occlusion, there was no passage of air. To eliminate any influence from the cricothyroid muscles or extrinsic laryngeal musculature, these muscles were severed bilaterally before the final assessment.

Electrical stimulation of the right PN was performed proximal to the anastomosis, and mobility and evoked activity were recorded using videolaryngoscopy and EMG.

The right PN-RLN anastomosis was fixed in situ, and removed for histological analysis. The animals were killed, and the larynx was excised. The right arytenoid was then palpated for signs of a reduced passive mobility of the cricoarytenoid joint compared with the left cricoarytenoid joint.

Videolaryngoscopic evaluation

The mobility of the reinnervated right hemilarynx was compared with the normal left hemilarynx. Abduction was scored as *good* if abduction on inspiration occurred almost synchronously with and equal in degree to the abduction of the left side; *adequate* if abduction on inspiration was more than half the abduction of the left side; *limited* if abduction on inspiration was half or less of the abduction of the left side; or *poor* if no effective or very slight abduction on inspiration was observed.

EMG evaluation

Electromyography was performed using transorally introduced hooked wire electrodes in the left and right PCA muscles, and in the left and right vocalis muscles (pars medialis of the thyroarytenoid muscle). An EMG type MS6 (Medelec, Old Walking, England) was used. Respiratory monitoring was performed simultaneously, using a custom-made impedance plethysmograph, registering chest movement.

Histological analysis

To obtain histological proof of the reinnervation, the nerve anastomoses were fixed in situ after the final assessment in the anesthetised animal using a cacodylate-gluteraldehyde solution and then resected for histological examination. After postfixation in the same solution, the tissue was embedded in epoxy resin, and 1µm thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5cm distal to the anastomosis, by computerized screening of a 500-µm-wide band across the widest nerve diameter, the axon count (axons per square millimeter) and the axon diameters were estimated.

Results

Evaluation was possible in 11 cats. One cat (cat 2) died during the delay.

Laryngeal abductor function assessment

(1) Before and after initial transection of RLN

Before transection, normal symmetrical abduction was seen on inspiration in all cats, and normal EMG activity was recorded in the PCA and vocalis muscles. Normally in the PCA muscle, there is a phasic inspiratory activity pattern, whereas the vocalis has only sporadic activity in rest, a burst of activity is seen during phonation and reflex glottic closure.

Immediately after transection of the right RLN, complete immobility of the right hemilarynx was seen and there was absence of EMG activity in the right vocalis and right PCA muscles in all cats.

(2) Before and after retransection of the RLN

Before retransection of the RLN, *limited* abduction mobility of the right hemilarynx was observed in 3 of the cats using videolaryngoscopy. The EMG recordings of the right PCA muscle showed signs of spontaneous subclinical reinnervation in 7 cats, in the other 4 cats no EMG activity was found. The EMG activity consisted of an inspiratory pattern (Figure 5.2a). After retransection of the distal RLN stump, ie, just before performing the delayed PN-RLN anastomosis, the right hemilarynx was immobile in all cats. EMG activity persisted in the right PCA muscle in 3 cats, consisting of a minor inspiratory activity pattern in 1 cat and an uncoordinated pattern in 2 cats (Figure 5.2b).The EMG recordings of the right vocalis muscles showed a minor inspiratory activity in 6 cats and an uncoordinated pattern of single motor unit potentials in 5 cats. In the 6 cats with minor inspiratory activity patterns in the vocalis muscle, EMG activity disappeared after retransection of the RLN (Figure 5.2b), whereas in the 5 cats with uncoordinated single motor units potentials, the activity persisted. The results of EMG of the PCA muscle and vocalis muscle are summarized in Table 5.1.

Table 5.1 EMG recordings in the right PCA and right vocalis muscles after 9-month delay

Cat no.	Before retransection of RLN		After retransection of RLN	
	PCA	Vocalis	PCA	Vocalis
1	0	1	0	0
3	0	S	0	S
4	1	1	0	0
5	1	1	0	0
6	1	1	S	0
7	1	S	1	S
8	0	S	0	S
9	0	1	0	0
10	1	1	S	0
11	1	S	0	S
12	1	S	0	S

**EMG indicates electromyographic; PCA, posterior cricoarytenoid; RLN, recurrent laryngeal nerve; I, irspiratory EMG activity; S, uncoordinated single motor unit potentials; and 0, no EMG activity*

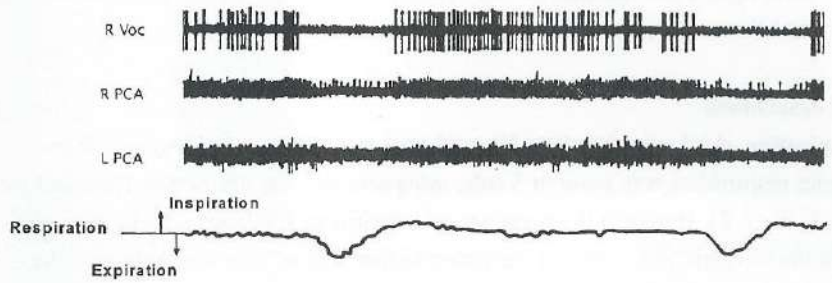


Figure 5.2a *Top, Electromyographic (EMG) recordings (2cm/s) 9 months after initial recurrent laryngeal nerve (RLN) transection. Spontaneously an inspiratory activity pattern has occurred in the right vocalis muscle (R Voc) and in the right posterior cricoarytenoid (R PCA)muscle. The pattern is almost synchronous with the normal inspiratory activity in the left PCA (L PCA) muscle. Bottom, The respiratory cycle.*

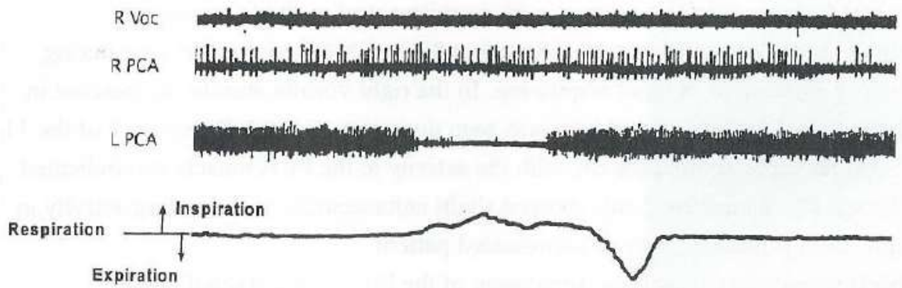


Figure 5.2b *Top, EMG recordings (2cm/s). After RLN retransection after the 9-month delay period, no EMG activity pattern is seen in the R Voc muscle and an uncoordinated pattern of single motor unit potentials is seen in R PCA muscle. In the L PCA muscle, normal inspiratory activity is seen. Bottom, The respiratory cycle.*

(3) Follow up after PN-transfer

First signs of PN activity in the EMG recordings, an inspiratory activity pattern starting about 30-40ms later than the normally innervated left PCA muscle, were recorded in the right PCA muscles at 4.8 weeks (±SD, 2.3 weeks). First signs of abduction mobility were seen at 6.7 weeks (±SD, 2.3 weeks).

(4) Final assessment

Quiet respiration. Abductor function 10 weeks after the reinnervation procedure during quiet respiration was *good* in 3 cats, *adequate* in 1 cat, *limited* in 1 cat and *poor* in 6 cats (Table 5.2). Between the presence of subclinical EMG activity in the right PCA after the 9 month delay period, recorded before and after retransection of the RLN and the final degree of abductor function recovery, Spearman rank correlations of 0.23 and 0.5 respectively, were found. Evaluation of EMG recordings showed typical PN phasic inspiratory activity in the right PCA muscle in 10 of the 11 cats (Figure 5.2c). In the remaining case (cat 8), the anastomosis was later found to have lost continuity and therefore considered a surgical failure. In the right vocalis muscle, a weak inspiratory EMG activity pattern was recorded in 9 of the 11 cats (Figure 5.2c). In the other 2 cats, uncoordinated series of single motor unit potentials were seen.

Respiratory distress. During respiratory distress the abductor function was good in 1 cat, adequate in 2 cats, limited in 3 cats and poor in 5. The 5 cats included the cat with surgical failure (cat 8). In 10 cats, the inspiratory EMG pattern in the right PCA increased during respiratory distress with recruitment of multiple motor unit potentials. In the cat with surgical failure (cat 8), no EMG activity was seen during respiratory distress, as in quiet respiration. In the right vocalis muscle, an increase in the inspiratory EMG activity pattern was seen during respiratory distress in 9 of the 11 cats. This occurred simultaneously with the activity in the PCA muscle and indicated synkinesis. The remaining 2 cats showed slight enhancement of the resting activity in the right vocalis muscle, with no coordinated pattern.

Electrical stimulation. Electrical stimulation of the PN nerve proximal to the anastomosis resulted in visible abduction or contraction of the right hemilarynx and evoked EMG activity in 10 of the 11 cats. In the cat with surgical failure (cat 8) there was no response to electrical stimulation.

Cricoarytenoid joint mobility. Palpation of the right arytenoid revealed reduced cricoarytenoid joint mobility in 6 cats. Five of these cats had a poor abductor function and 1 of them had limited abductor function. The 3 cats with good and the 1 cat with

adequate abductor function had a normal passive mobility of the cricoarytenoid joint. In 1 cat (cat 9), palpation of the arytenoid was not conclusive. Spearman rank correlation between restoration of function and normal passive mobility of the cricoarytenoid joint was 0.49.

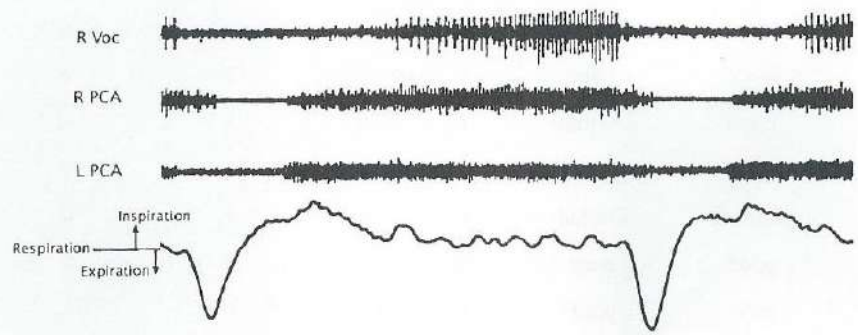


Figure 5.2c *Top, EMG recordings (2cm/s). Ten weeks after delayed selective reinnervation of R PCA muscle with phrenic nerve (PN), minor inspiratory activity is seen in the R Voc muscle, a typical PN inspiratory activity pattern in the R PCA and normal inspiratory activity in the L PCA. Bottom, The respiratory cycle.*

Histological analysis

Histological proof of reinnervation in the RLN was found in all 8 examined specimens. The specimen from the cat with surgical failure (cat 8) was excluded and specimens from the remaining 2 cats (cats 4 and 5) could not be evaluated due to an unsuccessful fixation procedure. The median axon count was 3771 axons/mm^2 with a mean axon diameter of 2.17µm. In one of our previous studies (Chapter 2) involving cats of the same breed, sex and weight range as were used in this study we found that the median axon count in the unaffected RLN was 3144/mm^2 and the mean axon diameter was 4.68µm. Analysis of variance indicates a significantly higher number and significantly thinner (regenerating) axons in the reinnervated RLN than in the normal RLN (p=0.009).

Table 5.2 Results of final assessment 10 weeks after reinnervation procedure

Cat no.	Abductor function: Quiet Respiration	Abductor function: Respiratory Distress	EMG activity: PCA	EMG activity: Vocalis	Electrical stimulation	Passive Mobility of CA Joint
1	poor	limited	1	1	1	R
3	good	adequate	1	1	1	N
4	poor	poor	1	1	1	R
5	good	adequate	1	S	1	N
6	good	good	1	1	1	R
7	limited	limited	1	1	1	R
8	poor	poor	0	S	0	R
9	poor	poor	1	1	1	---
11	adequate	limited	1	1	1	N
12	poor	poor	1	1	1	R
13	poor	poor	1	1	1	R

**EMG indicates electromyographic; PCA, posterior cricoarytenoid; CA, cricoarytenoid; 1, inspiratory; 0, no inspiratory; S, uncoordinated single motor unit potentials; R, reduced; and N, normal.*

Discussion

Selective reinnervation of the PCA muscle with a PN transfer has proved feasible even after a delay of 9 months. This was established by EMG PN activity in the PCA muscle in 10 of the 11 cats, PCA muscle contraction on electrical stimulation of the right PN in these 10 cats, and histological proof of reinnervation in examined nerve specimens in 8 cats. Even if subclinical reinnervation persisted following retransection of the RLN stump, it did not prevent surgical reinnervation in our study. A correlation is even suggested between persistence of subclinical reinnervation after retransection of the RLN and a good restoration of function (Spearman rank correlation coefficient: 0.50).

No histological examination of the muscular condition was performed, but no apparent denervation atrophy was observed during laryngoscopy or macroscopic postmortem examination. Probably the process of severe denervation atrophy had been prevented or reversed as a result of the subclinical reinnervation.

Despite evident reinnervation, however, the abductor mobility function following the delay was clearly less successful than after immediate reinnervation with the PN nerve transfer (Chapter 3&4). The results of our study are compared with previously reported results following immediate selective abductor reinnervation (Figure 5.3) during quiet respiration and respiratory distress. The main limiting factor in mobility restoration in our study appears to have been reduced passive mobility of the crico-arytenoid joint. In earlier studies (Chapter 2, 3 & 4) in which reinnervation was performed in the acute stage only 2 of 30 cats showed reduced passive joint mobility. Fixation of the cricoarytenoid joint due to fibrous ankylosis has occurred in rabbits starting at 5 months after RLN section, leading to complete fixation at 12 months (Langnickel 1973). In humans fibrous ankylosis due only to immobility after RLN paralysis appears to be exceptional (Elies et al. 1983). This difference is explained by lack of passive movement of the arytenoid owing to the lack of use of the vocal cords in animals (Elies et al. 1983).

Inappropriate reinnervation resulting in synkinesis may have been an additional limiting factor in the restoration of mobility in our study. All cats except that with surgical failure showed signs of inappropriate reinnervation of the right vocalis muscle with inspiratory EMG activity. Despite this fact, 3 cats achieved a good abductor function during quiet respiration. However, during respiratory distress, the abductor function decreased in 2 of these cats, although an enhanced inspiratory EMG activity pattern was observed in the PCA muscles. This can only be explained by inappropriate spontaneous reinnervation of the not surgically reinnervated vocalis muscles, resulting in synkinesis. Inspiratory EMG activity that was already present in the vocalis muscle during quiet respiration showed a marked increase during respiratory distress. This activity occurred simultaneously with the increased inspiratory activity in the PCA muscles during respiratory distress, impeding abductor function. In a previous study (Chapter 3) in which PN transfer was performed immediately following RLN transection, no inappropriate reinnervation of the vocalis muscle was observed, and respiratory distress condition resulted in an increased abduction.

The results obtained in our study cannot simply be translated to humans, but a number of factors are important to take into consideration when contemplating human reinnervation surgery. The best results of reinnervation surgery are obtained if the time interval between the injury and the time of reconstruction is short. Future research should be directed toward tests that can predict the chances of spontaneous nerve recovery, at an early stage, to select the patients with a poor prognosis of nerve recovery so they can benefit from reinnervation surgery. Although EMG data are believed to be reliable in predicting the spontaneous outcome of laryngeal paralysis (Thumfart 1988), conclusive evidence to support these claims is still lacking. Reinnervation surgery is likely to be beneficial especially for patients with bilateral

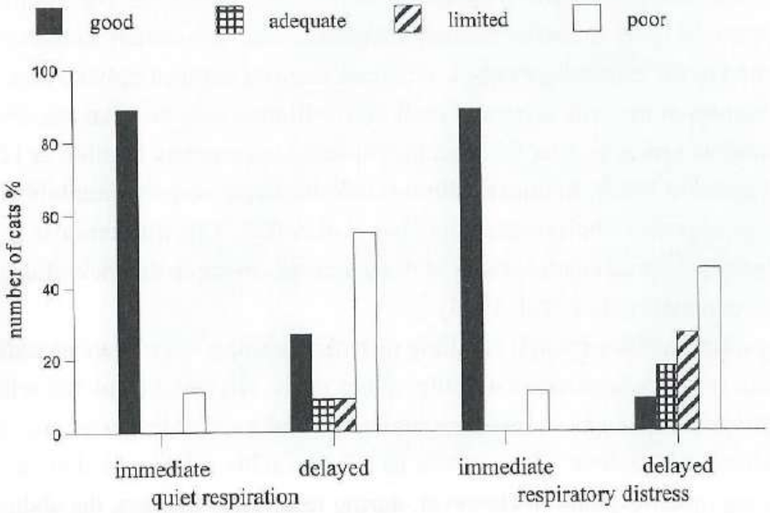


Figure 5.3 *Graph demonstrating the abductor function results after immediate (n=9) and delayed (n=11) selective abductor reinnervation with the phrenic nerve.*

paralysis. If there is certainty of loss of continuity of both RLNs, a unilateral reinnervation procedure using the PN may be considered immediately, as no spontaneous recovery can be expected following transection due to the inevitable inappropriate reinnervation and subsequent synkinesis as has been demonstrated in our previous study (Chapter 2).

For patients with unilateral paralysis and dysphonia, vocal fold augmentation or surgical medialization still appears superior to reinnervation surgery, and therefore early selection is less important in these cases.

In conclusion, reinnervation surgery may be considered immediately after severe bilateral nerve injury or in selected cases of long-standing bilateral RLN or vagus nerve paralysis where there is no hope for spontaneous recovery, provided there is normal cricoarytenoid joint mobility.

Chapter 6

Acute Recurrent Laryngeal Nerve Injury, an Indication for Immediate Nerve Repair?

Julie T van Lith-Bijl (MD), Hans F Mahieu (MD,PhD).

(submitted)

Abstract

Introduction In a unilateral recurrent laryngeal nerve (RLN) paralysis, voice problems due to incomplete glottic closure are predominant. As the final position of the paralytic vocal fold and degree of compensation of the unaffected vocal fold are unpredictable in the acute phase, permanent surgical medialization does not seem to be appropriate at this early stage. Laryngeal reinnervation procedures, if indicated, could, however best be performed in the acute phase. On the basis of review of the literature, a laryngeal reinnervation study in a cat model and our clinical experience, a management protocol for acute unilateral recurrent laryngeal nerve (RLN) injury is proposed.

Methods Adductor function recovery was evaluated in a cat model after RLN crush injury (N=20), nonselective RLN repair (N=10) and selective adductor reinnervation with the ansa cervicalis in combination with a selective abductor reinnervation with the phrenic nerve (PN) (N=10) and compared with a control group (N=11) in which the RLN was transected but not reconstructed. Using videolaryngoscopy and electromyography reinnervation was evaluated after a period of 10 weeks in the first three groups and after 9 months in the control group.

Results RLN crush injury resulted in restoration of normal adductor function in the majority of cats. Immobility of the reinnervated hemilarynx and inappropriate paradoxical movement was seen after nonselective nerve repair (simple reanastomosis). Selective abductor and adductor reinnervation resulted in restoration of muscle tonus without inappropriate paradoxical mobility. Protective reflex adduction to prevent aspiration was, however, not achieved. In addition two patient case reports are presented, and demonstrate paradoxical laryngeal activity after RLN-reanastomosis immediately following nerve transection.

Conclusions Based on our study and the review of the literature, selective adductor reinnervation with the ansa cervicalis seems to be the best reinnervation procedure in the acute phase after unilateral RLN injury, even though no restoration of reflex adduction will be achieved. Reanastomosis of the RLN must NOT be performed as this will lead to paradoxical mobility and severe functional impairment. Staged vocal fold medialization surgery is indicated if dysphonia resulting from insufficient glottic closure is still present after 6 to 9 months.

Introduction

Recurrent laryngeal nerve (RLN) or vagal nerve injury can result in a permanent disturbance of laryngeal mobility. In a unilateral paralysis, dysphonia due to incomplete glottic closure is predominant and depends on the position of the paralytic vocal fold and the compensatory adduction of the unaffected vocal fold across the midline. Incomplete glottic closure results in a breathy voice. Furthermore, loss of tonicity may result in an asymmetrical glottic cycle and diplophonia. In the case of a unilateral RLN paralysis respiratory and deglutitional problems are rare, but aspiration may occur. Problems of deglutition and aspiration are more common in unilateral vagal nerve paralysis.

These functional deficits are partly due to impaired laryngeal adduction, specifically the loss of reflex glottic closure, normally elicited by tactile stimulation of supraglottic mucosa, aimed at prevention of aspiration during the swallowing act. The protective reflex arc (Figure 6.1) is triggered by stimulation of tactile receptors in the glottic and supraglottic mucosa. The stimulus passes along the sensory fibers of the

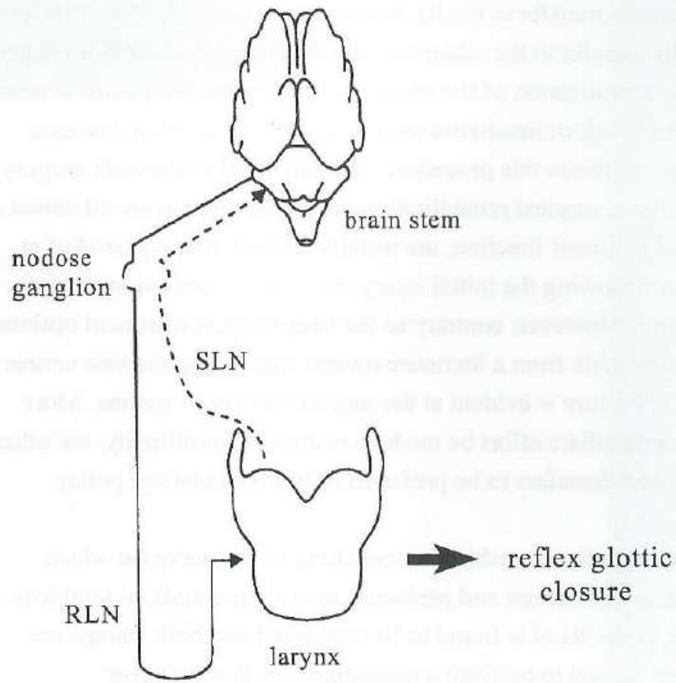


Figure 6.1 *Diagram showing laryngeal protective glottic closure reflex arch resulting from tactile supraglottic stimulation. SLN = superior laryngeal nerve; RLN = recurrent laryngeal nerve.*

internal branch of the superior laryngeal nerve (SLN) via the nodose ganglion to the nucleus solitarius, the nucleus of the SLN in the brain stem. There is a polysynaptic connection to the nucleus ambiguus and the motor fibers of the RLN, evoking contraction of the laryngeal adductor muscles and inhibition of the phasic inspiratory action potentials in the posterior cricoarytenoid PCA muscles (Kirchner 1991, Isogai et al. 1991, Suzuki et al. 1976).

Iatrogenic or traumatic injury to the RLN or vagal nerve is not always noticed at the time of surgery or trauma. Often the diagnosis is made much later, when the symptoms persist. Current surgical treatment options for incomplete glottic closure due to longstanding unilateral vocal fold paralysis consist of vocal fold augmentation by means of intracordal injection (Arnold 1962, Spiegel et al. 1987, Mikaelian et al. 1987); vocal fold medialization by means of laryngeal framework surgery (Isshiki et al. 1974, 1978, Mahieu et al. 1996), (usually considered a better option than intracordal injection); or reinnervation surgery, the use of which remains controversial (Crumley et al. 1986, Zheng et al. 1996, Tucker 1981). Laryngeal reinnervation procedures performed in cases with unilateral laryngeal paralysis consist of nonselective ansa cervicalis transfer to the RLN main stem (Crumley et al. 1986) or selective ansa cervicalis transfer to the adductor branch (Zheng et al. 1996). Tucker (1981) tried to achieve neurotization of the adductor muscles by using a nerve-muscle pedicle technique with a block of omohyoid muscle and its branch from the ansa cervicalis. He presently combines this procedure with laryngeal framework surgery (Tucker 1990, 1993). These surgical rehabilitation procedures which are all aimed at correction of the loss of adductor function, are usually applied after a period of at least 6 months to a year following the initial injury, to await possible spontaneous recovery or compensation. However, contrary to the later surgical treatment options no clear evidence is extractable from a literature review concerning the best course of action if a traumatic RLN injury is evident at the time of surgery or trauma. More specifically, should an immediate effort be made to restore nerve integrity, are other procedures including nerve transfers to be preferred or is a wait and see policy advisable.

RLN injury can range from crushing or stretching of the nerve (in which continuity of the axons, endoneurium and perineurium are preserved), to total loss of continuity of the nerve. If the RLN is found to be transected and both stumps are identified it would seem logical to perform a reanastomosis. It is however questionable whether this is the best treatment option, considering possible development of laryngeal synkinesis (Crumley 1979, Chapter 2). After reanastomosis

regenerating nerve fibers sprout and some may grow along paths to denervated muscles other than the original target muscle, a phenomenon known as misdirection of regenerating axons. For the RLN, misdirection is facilitated by the intraneural topography, abductor and adductor axons are intertwined and only divide into the subsequent branches intralaryngeally, close to their target muscles (Sunderland et al. 1952). Misdirected regeneration of these axons leads to inappropriate reinnervation of opposing muscle groups. The neural activity then causes simultaneous contraction (synkinesis) of adductors and abductors (Figure 6.2), resulting in inadequate mobility, immobility, or even paradoxical movement of the larynx. Laryngeal paradoxical movement implies abduction during phonation and adduction during inspiration or both.

As the final position of the paralyzed vocal fold and degree of compensation of the unaffected vocal fold are unpredictable in the acute phase, permanent augmentation and medialization methods of treatment do not seem to be appropriate at this early stage. In the case of crush injury a good chance of spontaneous recovery

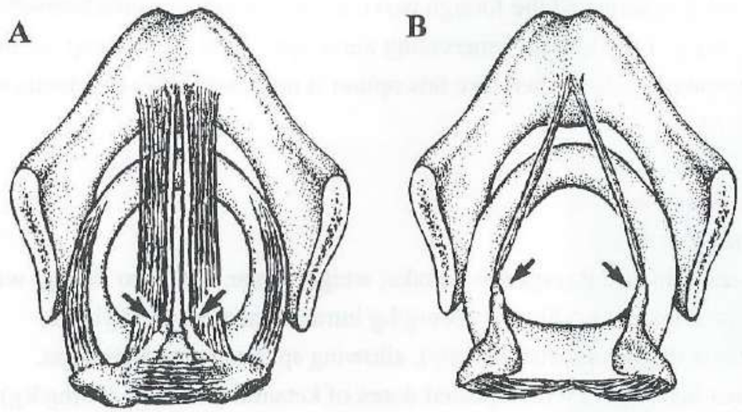


Figure 6.2 *A: contraction of the adductor muscles leads to adduction of the vocal folds (indicated by arrows), during phonation and reflex closure. B: contraction of the abductor muscle leads to abduction of the vocal folds (indicated by arrows), during the inspiratory phase of respiration.*

might be expected as long as continuity of the endoneurial sheath remains intact (Chapter 2, Boles et al. 1969). If there is loss of continuity of the nerve, spontaneous function recovery is unlikely. Regeneration of axons may lead to inappropriate reinnervation and synkinesis. In the case of loss of continuity at the acute stage there appear to be four treatment options: (1) nonselective reinnervation (RLN-reanastomosis) with repair of the transected nerve ends; (2) nonselective anastomosis of a foreign nerve transfer to the distal RLN stump; (3) selective reinnervation with a foreign nerve anastomosis to the adductor branch with or without additional selective abductor reinnervation; (4) await spontaneous compensation with correction of insufficient glottic closure at a later stage if required.

In order to determine the most appropriate mode of management we investigated the laryngeal adductor function recovery in a cat model after crush injury, nonselective RLN repair and selective adductor reinnervation using the ansa cervicalis in combination with a selective abductor reinnervation using the phrenic nerve and compared the results with a control group in which the RLN mainstem was transected and not reconstructed.

The second nonselective option of anastomosing a foreign nerve to the distal RLN stump seems to be the poorest option in the above described situation, as it would add loss of function of the foreign nerve to only achieve a nonselective anastomosis, while the originally innervating nerve (proximal RLN stump) is also available for reanastomosis. Therefore this option is not taken into consideration in our study.

Materials and methods

Surgical method

Fifty-one female cats (aged: 6 months; weight range: 2200g to 2800g) were anesthetized with ketamine chloride (20mg/kg intramuscularly) and xylazine hydrochloride (0.5mg/kg subcutaneously), allowing spontaneous respiration. Anesthesia was maintained with repeated doses of ketamine chloride (10mg/kg) every 10-20 minutes.

The following procedures were performed each in a separate group of cats: (1) crush injury to the RLN, (2) repair of the transected RLN and (3) selective reinnervation of the laryngeal adductor and abductor muscles, (4) the RLN was transected and not surgically reinnervated.

A midline incision of the neck was performed. Pretracheal muscles were separated in the midline and the proximal part of the trachea was exposed. The right RLN was then identified.

In 20 cats a crush injury of the right RLN was induced (Figure 6.3). At a distance of 2.0cm caudally from the cricothyroid joint the nerve was crushed for 30 seconds with hemostatic forceps with grooved jaws (Halstead No. 02401.12, manufacturer: Simal, Mariakerke, Belgium) by closing the forceps for three clicks (Chapter 2). Immediately after crushing, the distal point of the crush was marked with a suture in the perineurium.

In 10 cats the right RLN was sectioned at a distance of 2.0cm caudally from the cricothyroid joint (Figure 6.3). The ends were then reapproximated with an 10-0 microsuture (Ethilon, Ethicon Inc, Somerville, NJ) and fixed with fibrin glue (Tissucol, Austrian Institute for Hemoderivates, Vienna, Austria). An additional suture was placed adjacent to the site to facilitate reidentification.

In 10 cats the right RLN and right ansa cervicalis were identified. The latter was followed to its sternohyoid branch and transected near the sternohyoid muscle.

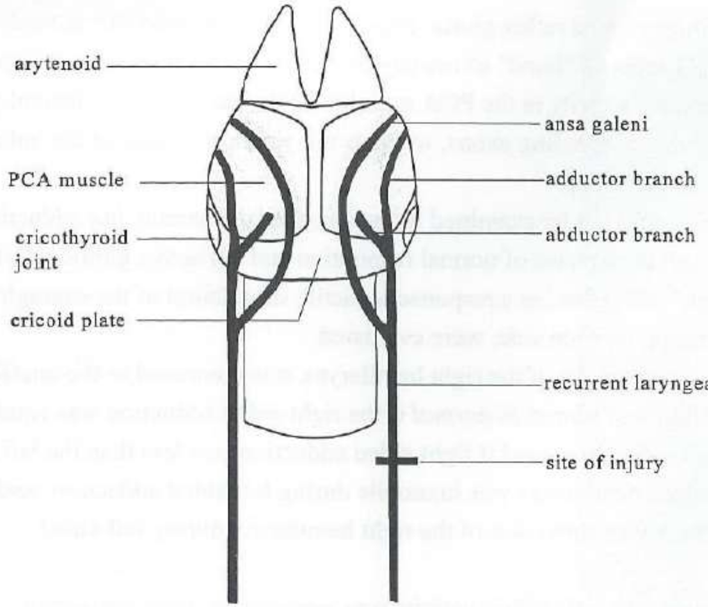


Figure 6.3 *Site of crush injury and transection, dorsal view of larynx.*

The proximal stump was then transferred and anastomosed to the distal stump of the adductor branch of the RLN using a microsuture and fibrin glue, having transected the adductor branch just distal to the abductor branch. The procedure was combined with a selective reinnervation of the abductor muscle using a phrenic nerve (PN) transfer to the RLN main trunk, as described in previous studies (Chapter 3 & 4) (Figure 6.4). In our present study only the adductor function is evaluated.

In the control group, the right RLN was exposed in 11 cats and transected at a distance of 2.5cm from the cricothyroid joint. To prevent early spontaneous restoration of continuity, 4-5cm of the proximal part of the right RLN was resected.

Assessment of Laryngeal Adductor Function

Laryngeal mobility was recorded by videolaryngoscopy. Normally in cats, (Chapter 2, 3 & 4) as in humans, there is abduction of the vocal folds during inspiration followed by an adduction movement back to the paramedian position during expiration. During respiration an inspiratory EMG activity pattern is observed in the PCA muscles but no particular pattern is seen in the vocalis muscle, suggesting that the adduction movement during expiration may be a result of passive elastic forces.

During phonation or reflex glottic closure the vocal folds adduct actively to the midline and EMG shows a "burst" of activity in the vocalis muscles and inhibition of the phasic inspiratory activity in the PCA muscles. In the cat there is a bilateral glottic closure response due to crossing axons, whereas this reflex is strictly unilateral in humans (Sasaki et al. 1976).

Phonation could not be examined in the anesthetized animal, but adduction during (1) the expiratory phase of normal respiration and (2) active glottic reflex closure or "reflex" adduction, as a response to tactile stimulation of the supraglottic laryngeal mucosa on the right side, were evaluated.

Adduction movement of the right hemilarynx was compared to the unaffected left side. Adduction was scored as *normal* if the right-sided adduction was equal to or more than the left side; *decreased* if right-sided adduction was less than the left; *immobile* if the right hemilarynx was immobile during left-sided adduction; and *paradoxical* if there was abduction of the right hemilarynx during left-sided adduction.

Electromyographical (EMG) activity was recorded by using transorally introduced hooked wire electrodes and an EMG type MS6 (Medelec, Old Walking, England). The EMG activity was recorded of the left and right posterior

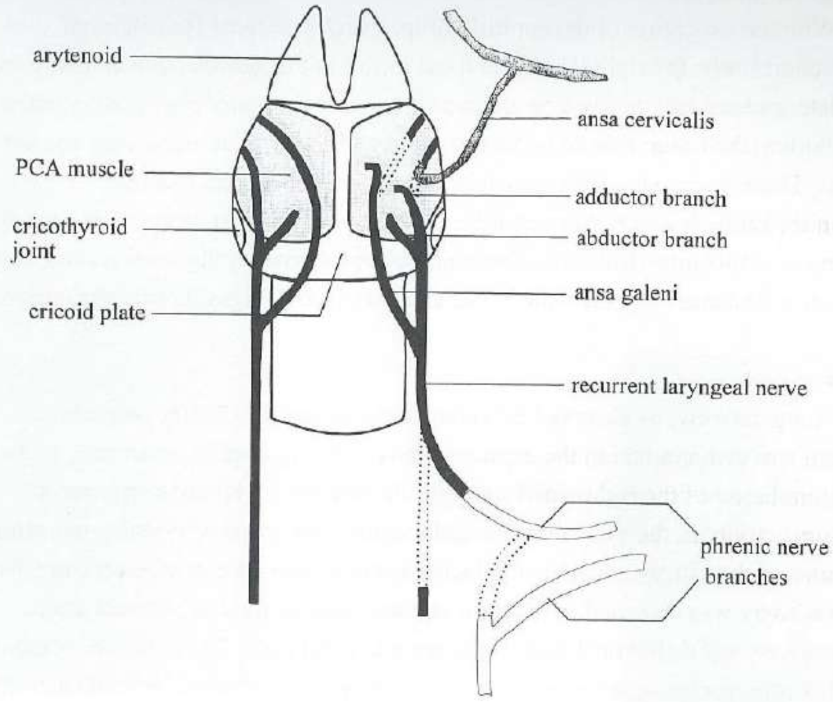


Figure 6.4 *Selective adductor and abductor reinnervation procedure with ansa cervicalis anastomosis to the adductor branch and phrenic nerve to main recurrent laryngeal nerve trunk.*

cricoarytenoid (PCA) muscles, and the left and right vocalis muscles (the medial part of the thyroarytenoid muscles). Respiratory monitoring was performed simultaneously using a custom-made impedance plethysmograph registering chest movement.

Laryngeal adductor function was assessed preoperatively, immediately postoperatively and after a ten week follow-up period using videolaryngoscopy and EMG. At 10 weeks, during final assessment after reinnervation, the strap muscles and the cricothyroid muscles were transected to eliminate their influence on vocal fold adduction. Thereafter electrical stimulation of the nerves involved was performed proximal to the site of injury.

In the control group in which the RLN was transected but not reconstructed laryngeal function assessment was performed only after a 9 month period. Since no site for electrical stimulation could be identified proximal to the injury site, reaction to electrical stimulation was not assessed in the control group.

Histological Analysis

With the exception of the control group, which was used for a delayed reinnervation study, the right RLN was fixed in situ in the anesthetized animal using a cacodylate-gluteraldehyde solution and was then resected. After postfixation in the same solution, the tissue was embedded in epon and 1-µm-thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5cm distal to the anastomosis. By computed screening of a 500-µm-band across the widest nerve diameter, the axon count (axons per square millimeter) was estimated. The cats were sacrificed after this evaluation.

Results

Preoperatively, as observed by videolaryngoscopy, in all fifty-one cats adduction was symmetrical in the expiratory phase during normal respiration and on tactile stimulation of the right-sided supraglottic mucosa. EMG showed normal inspiratory activity in the PCA muscles and a minor activity pattern with some single motor units in the vocalis muscles. On tactile stimulation of the supraglottic mucosa, a burst of activity was recorded in the right and left vocalis muscles. Immediately postoperatively the right vocal fold was immobile in all cases. Denervation of the right RLN after each surgical procedure was confirmed by absence of EMG activity in the right vocalis muscle and the right PCA muscle. The results of evaluation of the adductor function 10 weeks after each surgical procedure, and 9 months after resection in the control group, are presented in Table 6.1. A further explanation of the results is given below.

Adductor function after crush injury

Ten weeks after crush injury of the right RLN videolaryngoscopic evaluation showed that adduction during normal respiration after crush injury was *normal* in 18 cats (in one cat adduction of the right side even exceeded that on the left); and in 2 cats adduction on the right was *decreased*.

No EMG activity was seen in the right vocalis muscle, in 18 cats during normal respiration, and expiratory activity was recorded in the right vocalis muscle in 2 cats (one of these had decreased adduction movement). During normal respiration no EMG activity was recorded in the unaffected left vocalis muscles in any of the cats. In the right and left PCA muscles an inspiratory activity pattern was recorded in all cats.

The reflex adduction was *normal* in 19 cats (videolaryngoscopy) in 1 of which right-sided adduction even exceeded that on the left side and in 1 cat there was *decreased* adduction on the right side (this cat also had decreased adduction during normal respiration and expiratory EMG activity in the right vocalis muscle).

Tactile stimulation of the right-sided supraglottic mucosa resulted in a burst of EMG activity in the right vocalis muscle in all 20 cats as well as in the 20 left vocalis muscles, but did not result in activity in the right or left PCA muscles.

Table 6.1 Laryngeal adductor function results of the four reinnervation conditions

Adduction Mobility		Crush n=20	Non-selective reinnervation n=10	Selective reinnervation n=9	RLN transected n=11
Normal Respiration	normal	18	0	6	0
	decreased	2	5	2	3
	immobile	0	3	1	8
	paradoxical	0	2	0	0
Reflex Adduction	normal	19	0	0	1
	decreased	1	3	2	1
	immobile	0	0	7	8
	paradoxical	0	7	0	1

Reflex adduction indicates adduction evoked by stimulation of supraglottic mucosa; Non-selective reinnervation, reanastomosis of the recurrent laryngeal nerve mainstem; Selective reinnervation, ansa cervicalis anastomosis to the adductor branch; RLN transected, the nerve was cut and not reconstructed during a period of 9 months.

Adductor function after non-selective RLN repair

Ten weeks after nonselective RLN repair videolaryngoscopy during normal respiration showed *decreased* adduction of the right hemilarynx in 5 cats, and the right vocal fold was *immobile* in 3 of the 10 cats. In 2 cats there was *paradoxical* movement, the right vocal fold abducting during adduction of the left vocal fold.

EMG of the right vocalis muscle, during normal respiration showed no activity in 1 cat, and an inspiratory activity pattern in 9 cats. In the left vocalis muscles, during normal respiration, no activity was found in 9 cats; and an expiratory activity pattern in 1 cat. In both the left and right PCA muscles synchronous inspiratory activity patterns were recorded in all 10 cats.

Videolaryngoscopic evaluation on tactile stimulation of the right-sided supraglottic mucosa resulted in *decreased* reflex adduction on the right side in 3 cats and *paradoxical* reflex abduction on the right side in 7 cats. Normal reflex adduction of the left hemilarynx was seen in all cats.

In response to tactile stimulation of the right-sided supraglottic mucosa, a burst of EMG activity was recorded in the right vocalis muscle in 8 cats, and no increase of activity in 2 cats. In the left vocalis muscle there was a burst of EMG activity in all cats. A simultaneous burst of EMG activity was found in 9 cats in the right PCA muscle as well. No increase of EMG activity was recorded in any of the cats concerning the unaffected left PCA muscle.

Adductor function after selective reinnervation of the laryngeal adductor and abductor muscles

In 1 cat iatrogenic damage to the right RLN occurred before performance of the PN-RLN anastomosis, precluding successful reinnervation. This case was excluded from further evaluation. The other 9 cats could be evaluated. Ten weeks after selective adductor and abductor reinnervation, videolaryngoscopy during normal respiration showed *normal* right-sided adduction in 6 of the 9 cats. In 2 there was *decreased* adduction movement, and in one cat the right vocal fold remained *immobile* throughout the respiratory cycle. In the latter case reduced passive mobility of the cricoarytenoid joint was found on palpation, which was probably due to fibrosis following the reinnervation surgery.

In 7 right vocalis muscles a weak inspiratory EMG pattern typical for the ansa cervicalis was recorded, comprising a weak inspiratory pattern which started later than that in the left or right PCA muscles and usually consisted of a single motor unit potential. In 2 cats there was no spontaneous activity in the right vocalis muscle.

Videolaryngoscopic evaluation on tactile stimulation of the right-sided supraglottic mucosa resulted in *decreased* right-sided reflex adduction in 2 cats and *immobility* of the right hemilarynx in 7 cats, whereas normal reflex adduction was seen in all cats on the left side.

No EMG activity was recorded in the right vocalis muscles, in response to the tactile mucosal stimulation, in any of the cats, whereas a burst of EMG activity was recorded in the left vocalis muscles in all cats. The left and right PCA muscles showed no increase in activity in any of the cats following tactile mucosal stimulation.

Adductor function after RLN transection

In the control group, 9 months following resection of the right RLN without reconstruction, *decreased* adduction mobility of the right hemilarynx was observed during normal respiration in 3 of the cats with videolaryngoscopy, in the other 8 cats the right vocal fold was *immobile*.

EMG recordings of the right vocalis muscle showed a minor inspiratory activity in 6 cats and an uncoordinated pattern of single motor unit potentials in 5 cats. EMG recordings of the right PCA muscle showed signs of spontaneous subclinical reinnervation in 7 cats, in the other 4 cats no EMG activity was found in the right PCA muscle. The EMG activity consisted of an inspiratory pattern.

Videolaryngoscopic evaluation on tactile stimulation of the right-sided supraglottic mucosa resulted in *normal* right-sided reflex adduction movement in 1 cat, *decreased* adduction in 1 cat and *immobility* of the right hemilarynx in 8 cats. In 1 cat *paradoxical* abduction movement was seen on the right side. *Normal* reflex adduction was seen in all cats on the left side.

EMG activity was observed only in the cat with *decreased* adduction in both the right vocalis and PCA muscles, in response to tactile supraglottic stimulation, whereas a burst of EMG activity was found in the left vocalis muscle in all cats.

Electrical stimulation

Electrical stimulation proximal to the anastomosis or injury site, was performed in 19 cats after crush injury, 9 after reanastomosis and 9 after selective adductor reinnervation. In all cases a contraction of the hemilarynx concerned was observed on videolaryngoscopy, also in the cat with reduced mobility of the cricoarytenoid joint, verifying that axon regeneration had taken place across the anastomosis.

Histological analysis

Histological analysis was performed on 19 nerve specimens after crush injury and on 9 specimens after RLN repair. In both groups regenerated axons were encountered, the number of axons per square millimeter did not differ significantly. After the selective adductor reinnervation procedure the ansa-adductor branch anastomosis nerve specimens could not be examined successfully due to fibrosis around the anastomosis site and the small caliber of the nerve anastomosis involved. The PN-RLN anastomosis for the abductor reinnervation in this group showed successful regeneration. The histological results of the latter specimens have been documented and discussed in our previous paper on laryngeal abductor function restoration (Chapter 4). Histological analysis was not perfomed in the control group.

Patient Case Histories

PATIENT 1

A 52 year old male was referred to our hospital with severe dysphonia. He had undergone left-sided thyroidectomy because of a goitre 10 months previously. During the surgical procedure the left RLN had been accidentally cut, its ends identified and reanastomosed. Postoperatively he had no complaints of dyspnea, but a severe dysphonia persisted.

On videolaryngostroboscopic examination we saw a dysfunction of the left hemilarynx and normal mobility of the right hemilarynx. During inspiration a short paradoxical adduction movement was seen of the left arytenoid. During phonation the left hemilarynx remained immobile in a lateral position, leaving a large glottic gap. The maximal phonation time was 5 seconds.

To medialize the left vocal fold a thyroplasty Type 1 and arytenoid adduction procedure (Isshiki et al. 1974, 1978) with fixation of the cricoarytenoid joint were performed on the left side under local anesthesia. Good voice quality resulted. On videolaryngostroboscopy a good glottic closure was seen during phonation and the maximal phonation time had increased to 11 seconds.

PATIENT 2

A 53 year old female patient presented with complaints of dyspnea during physical exertion. Her voice was dysphonic but functionally acceptable when speaking normally. When attempting to phonate loudly the voice deteriorated. Two years and 2 months earlier she underwent a right-sided thyroidectomy for treatment of a goitre. During the thyroidectomy the right RLN was accidentally severed and reanastomosed.

Videolaryngostroboscopy demonstrated normal function of the left hemilarynx and dysfunction of the right hemilarynx. During normal respiration the right hemilarynx was immobile but the glottic aperture was sufficient. During forced inspiration slight paradoxical adduction movement was seen of the right hemilarynx, whereas the normal left side abducted. Whilst phonating the right hemilarynx remained immobile in an intermediate position and incomplete closure of the glottis was seen over the whole length of the vocal folds. During loud phonation the glottic gap remained unchanged despite the increased subglottic pressure associated with louder phonation which usually decreases the incomplete closure. Spirometrie

revealed a normal vital capacity and a mildly restricted inspiratory cycle of the flow-volume curve.

Considering the acceptable voice quality during normal speech and the mildness of the dyspnea no further action was undertaken. In case of severe dyspnea retransecting the RLN would have been considered.

Discussion

As the condition of obvious acute injury to the RLN is rare, no large series of patients have been published from which a mode of management can be derived. Therefore we have attempted, on the basis of our animal experiments, the 2 patient histories, our own experience on treatment of unilateral vocal fold paralysis and review of the literature, to form a management protocol which is summarized in the algorithm in Figure 6.5.

Our results show that after RLN crush injury the adductor function usually recovers. These observations are in agreement with the generally held belief in clinical practice, that recovery of function is the rule after crush injury to the nerve. If these findings can be directly translated to the situation in humans, no specific treatment will be required if the nerve is crushed during trauma or has accidently been caught in a hemostat during surgery.

In the case of loss of continuity of the RLN, reanastomosis of both stumps, although tempting, appears to be contraindicated seeing the poor results in our study. The poor results can be explained by synkinesis of abductor and adductor muscles as is proven by simultaneous EMG activity of these opposing muscle groups. This synkinesis can even result in paradoxical mobility which can severely impair all laryngeal functions. In our experiment, paradoxical movement of the right hemilarynx was seen, especially on tactile stimulation of the supraglottic mucosa, so that glottic reflex opening resulted instead of reflex closure, subsequently increasing the risk of aspiration whereas paradoxical mobility could only be demonstrated in 1 cat after a prolonged period of time after transecting the RLN without surgical reconstruction.

In patients severe damage to the RLN without reconstruction invariably results in permanent immobility of the hemilarynx concerned. The 2 patients who underwent RLN-reanastomosis during thyroidectomy, both showed signs of synkinesis, paradoxical adduction during inspiration and dysfunctional immobility of the hemilarynx concerned during phonation.

Since reanastomosis of the RLN leads to paradoxical mobility with poor functional results the only sensible option when the RLN (or VN) is found to be

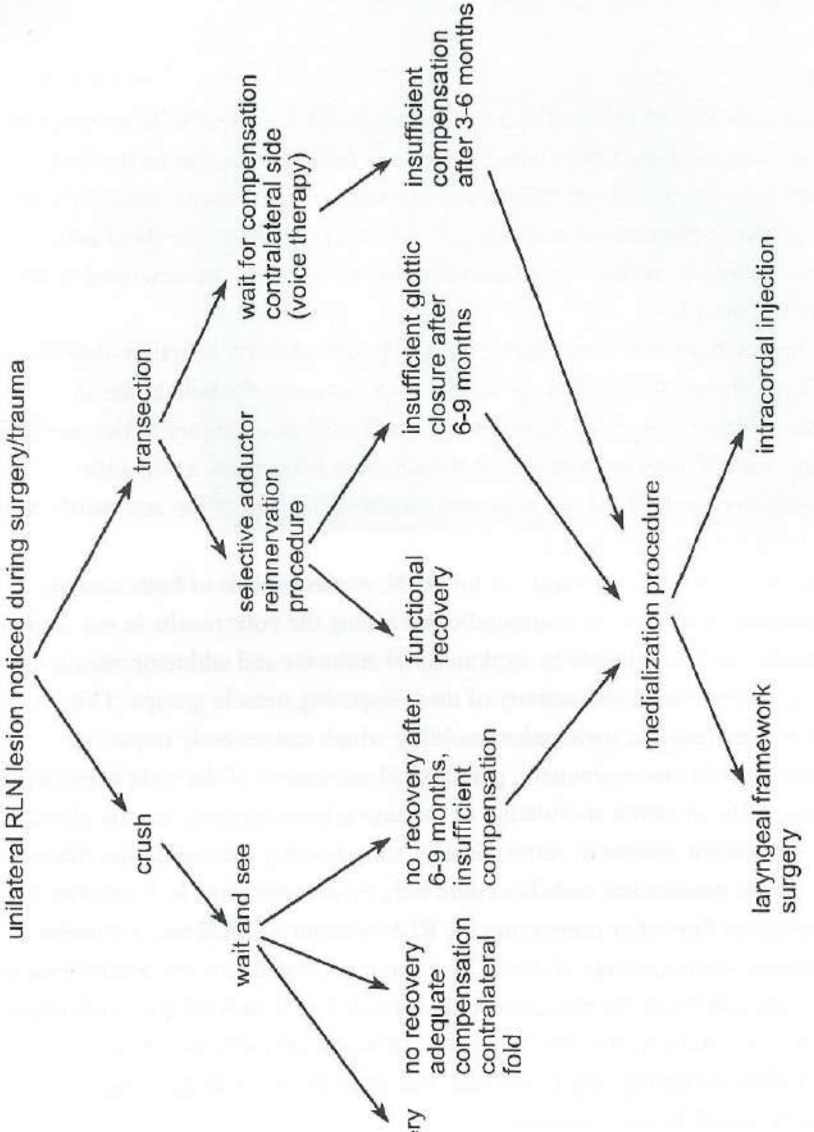


Figure 6.5 *Management algorithm for acute unilateral recurrent laryngeal nerve paralysis*

transected is therefore, either to wait and see or to perform a selective reinnervation procedure. If no immediate action is undertaken voice therapy can be initiated to assist compensation of the contralateral vocal fold and prevent improper compensatory mechanisms, such as ventricular phonation. It remains controversial whether voice therapy actively stimulates compensation of the contralateral vocal fold or whether this compensation occurs spontaneously anyway. In our department 3 to 6 months is usually awaited for compensation to become effective. If there is sufficient glottic closure and adequate phonation 3 to 6 months after RLN injury, no further action is required. If however, there is still no noticeable compensation 3 to 6 months following known RLN discontinuity a medialization procedure of the paralyzed vocal fold is indicated, consisting of laryngeal framework surgery (Isshiki et al. 1974, 1978, Mahieu et al. 1996), as the first treatment of choice or augmentation by means of intracordal injection as an alternative (Arnold 1962, Spiegel et al. 1987).

Non-selective anastomosis of the ansa cervicalis branch to the RLN main stem as is suggested by Crumley (1986) will of course also lead to synkinesis of the abductor and adductor muscles and therefore will not result in restoration of function. The tonicity achieved, however, in the absence of paradoxical mobility may be beneficial to phonatory function.

A selective reinnervation procedure such as anastomosis of a branch of the ansa cervicalis to the adductor branch of the RLN may be considered in the acute phase of RLN with loss of continuity of the nerve. An additional anastomosis of the phrenic nerve to the laryngeal abductor muscle, however, only seems to be indicated in cases of bilateral transected RLNs because usually in unilateral paralysis, the airway remains sufficient. Our results show that selective reinnervation of the vocalis muscle and thus restoration of tonicity is achieved but that reflex adduction is not restored, since the ansa cervicalis is not involved in the reflex arc (Figure 6.1).

If a reinnervation procedure has been performed, a recovery period of at least 6 months must be taken into account (Crumley 1979). During the initial period there will probably be considerable dysphonia with its accompanying morbidity. Voice therapy is optional. If after a period of 6 to 9 months there is still insufficient glottic closure an additional surgical medialization procedure can be taken into consideration.

Based on our results in the animal model, the best laryngeal reinnervation procedure in the acute phase after unilateral RLN section in patients, seems to be, selective adductor anastomosis with the ansa cervicalis in order to restore tonicity of the vocal fold, even though no restoration of reflex adduction will be achieved. Reanastomosis of the transected stumps of the RLN must NOT be performed at the acute stage as this will lead to paradoxical mobility in the majority of cases. Staged medialization surgery is indicated if insufficient glottic closure is still present after 6 to 9 months.

Chapter 7

Reinnervation Aspects of Laryngeal Transplantation

Julie T van Lith-Bijl (MD), Hans F Mahieu (MD,PhD).

European Archives of Otolaryngology 1998 (accepted for publication)

Abstract

Introduction Restoration of laryngeal function after laryngeal transplantation depends on appropriate reinnervation. Non-selective reinnervation procedures result in synkinesis and poor function restoration. This study was performed to test the feasibility of selective reinnervation procedures to restore laryngeal function.
Materials and methods Three surgical reinnervation procedures were studied each in a group of 10 cats. In group 1 a non-selective procedure in which the recurrent laryngeal nerve (RLN) was cut and reanastomosed. In group 2 selective abductor reinnervation was performed with the phrenic nerve. In group 3 selective abductor reinnervation with the phrenic nerve (PN) was combined with selective adductor reinnervation with the ansa cervicalis. Ten weeks after surgical reinnervation abductor and reflex adductor function was evaluated with videolaryngoscopy and EMG.
Results Nonselective reinnervation not only gave poor abduction during inspiration but even resulted in paradoxical movement during reflex adduction. Selective abductor reinnervation resulted in good abductor function. Selective adductor reinnervation with the ansa cervicalis brought about muscle tonus but no restoration of reflex adduction. Enhanced activity during respiratory distress caused only slight compromise of the abductor function.
Conclusions Selective laryngeal reinnervation with the PN and ansa cervicalis results in a good restoration of the respiratory laryngeal function. Concerning deglutition following laryngeal motor and sensory reinnervation, protection of the respiratory tract may not however be sufficient as no reflex glottic closure is achieved. More research is required.

Introduction

Total laryngectomy as is often performed in advanced laryngeal cancer is generally considered to be a severely debilitating surgical procedure due to the aphonia produced (McNeil et al. 1981). Therefore alternatives have been sought. These include (1) subtotal laryngectomy, indications being limited to specific tumor extensions and resulting in less than optimal laryngeal function concerning voice, respiration and deglutition; (2) creation of an artificial larynx which is still in an early stage of development; and (3) laryngeal transplantation.

Experimental laryngeal transplantation has been performed in several animal investigations using rats and dogs (Ogura et al. 1966, Silver et al. 1970, Berke et al. 1993, Anthony et al. 1995, Strome et al. 1992, Weed et al. 1995). The only reported

uman laryngeal transplantation by Kluyskens in 1970 was no success and led to nany critical comments. Longstanding viability of a properly functioning larynx has ıot yet been achieved in an animal model. For laryngeal transplantation to succeed hree basic problems have to be solved: patent vascular anastomoses, prevention of graft rejection and functional reinnervation.

The first of these issues, has been solved in the canine model (Ogura et al. 966, Silver et al. 1970, Berke et al. 1993). Laryngeal viability has been obtained with eestablishment of the arterial blood supply through a unilateral common carotid ınastomosis proximal to the superior thyroid branch. In clinical practice with urrently available modern microsurgical techniques reanastomosis of the superior hyroid artery or even the superior laryngeal artery, which normally supplies ıpproximately 80% of the blood to the larynx, could be used. Unilateral external ugular vein anastomosis including the hyoid venous arcade can be and has been used o reestablish a patent venous system.

The issue of graft rejection seems to be a more difficult problem, especially ince the potential recipient may well be an oncological patient, who is considered to equire an unimpaired immunological system to fend off metastatic disease. The esson from the past concerning the only patient who actually did undergo laryngeal ransplantation, involved his succumbing to generalized metastatic disease attributable o immunosuppression. As a consequence, this matter should be taken to heart Kluyskens 1970). However, progress in the field of transplantation in general, specially concerning the development of new selective immunosuppressive drugs, is promising in this respect (Strome et al. 1991). Liver transplants have now been performed successfully in patients for both primary and metastatic tumors (Starzl et al. 1989). Furthermore, one should realize that graft rejection of a larynx is not now the fatal condition that it is in many other fields of transplantation. This might allow for a ess aggressive immunosuppressive approach in laryngeal transplantation.

Restoration of functional laryngeal reinnervation is the remaining aspect which nust yet be adequately solved before laryngeal transplantation becomes feasible in ıumans. The normal larynx serves three functions. In order of priority for restoration hese functions are: sufficient airway for respiration by means of phasic active ıbduction of the vocal folds during inspiration; protection of the respiratory tract during swallowing by reflex glottic closure; and phonation by voluntary adduction.

Efforts to functionally reinnervate the larynx by means of anastomosis of the recurrent laryngeal nerve (RLN) mainstem are doomed to fail because of the synkinesis that will develop in the opposing laryngeal muscle groups (Chapter 2). If the separate abductor and adductor branches could be preserved and anastomosis of the separate branches and/or a nerve muscle pedicle for the abductor (Tucker 1974) could be performed, motor reinnervation and adequate laryngeal function could be achieved (Berke et al. 1993, Anthony et al. 1995), However, oncological principles, cannot be violated.

In most laryngeal malignancies and the occasional laryngeal trauma case in which the larynx cannot be preserved due to oncological safety margins or fibrosis, the separate RLN branches are not available for anastomosis. In such cases the RLN will also have to be removed. Therefore alternative reinnervation procedures must be sought such as separate selective motor reinnervation of the opposing laryngeal muscle groups (Chapter 3 & 4, Marie et al. 1989). Restoration of laryngeal abductor respiratory function remains the main priority, followed by reflex glottic closure adductor activity, in order to prevent aspiration during swallowing. Phonatory function does not necessarily require active adduction, as long as both vocal folds are more or less in the median or paramedian position during phonatory effort. Current experience has shown that restoration of active voluntary adduction appears to be of least importance and was not specifically studied at this time. Our present study objective was to look into the feasibility of restoring laryngeal motor function with a surgical reinnervation technique. For this purpose an experimental animal model in cats was used.

Materials and methods

Thirty cats (female, age, 6 months; weight range, 2200-2800 g) were anesthetized with ketamine chloride (20mg/kg given intramuscularly) and xylazine hydrochloride (0.5mg/kg given subcutaneously), allowing spontaneous respiration. A midline incision of the neck was performed. Pretracheal muscles were separated in the midline, and the upper part of the trachea was exposed. The right RLN was then identified and transected followed by one of three surgical reinnervation procedures (Figure 7.1). The left RLN served as the normal control in each cat.

Ten cats (group 1) underwent a reanastomosis of the sectioned right RLN. At a distance of 2.0cm from the cricothyroid joint the right RLN was cut and the ends reanastomosed using one approximating 10-0 microsuture (Ethilon, Ethicon Inc, Somerville NJ) and fibrin glue (Tissucol, Austrian Institute for Haemoderivates, Vienna, Austria).

Ten cats (group 2) underwent a selective abductor reinnervation with a phrenic nerve (PN) transfer. Since the cat has two PN branches on each side originating from

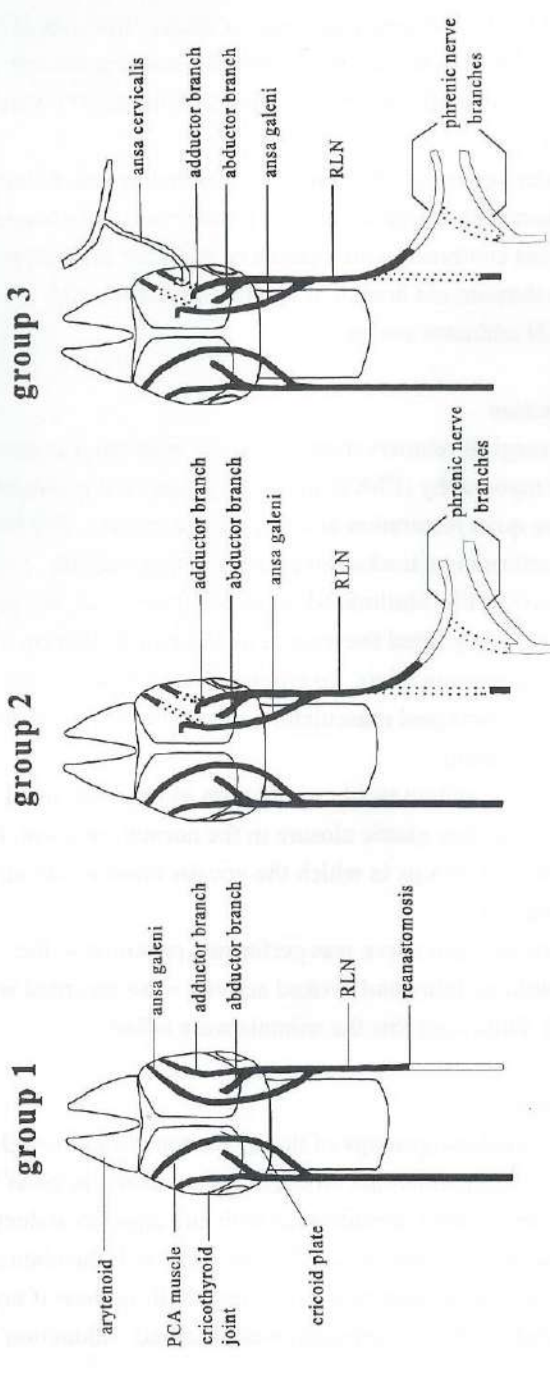


Figure 7.1 *Schematic representation of surgical reinnervation procedures, dorsal view of larynx. Group 1: reanastomosis of recurrent laryngeal nerve (RLN); group 2: abductor reinnervation with the phrenic nerve (PN); group 3: adductor and abductor reinnervation with the ansa cervicalis and PN respectively.*

the cervical roots C5 and C6, the C5 PN branch was dissected and anastomosed to the distal stump of the transected RLN. The ansa galeni and adductor branches of the RLN were transected and the proximal stumps were sutured into the posterior cricoarytenoid (PCA) muscle, so that all reinnervating axons from the PN were directed into the PCA muscle.

Ten cats (group 3) underwent reinnervation of both adductor and abductor muscle groups. The above described procedure using a phrenic nerve to reinnervate the abductor (PCA muscle) was combined with a selective adductor reinnervation with the ansa cervicalis. The sternohyoid branch of the ansa cervicalis was anastomosed to the distal RLN adductor stump.

Assessment of laryngeal function

Ten weeks after each surgical reinnervation, laryngeal function was assessed by means of laryngeal electromyography (EMG) and videolaryngoscopy. Abductor function was evaluated during quiet respiration and respiratory distress. The latter condition was achieved by performing a tracheotomy and occlusion of the tracheotomy tube (type Shiley 00 CFS; Mallinkrodt Medical, Irvine, CA, USA.) for 1 minute. The tracheotomy tube closely fitted the trachea in all cases so that on occlusion there was virtually no passage of air. To eliminate any influence from the cricothyroid muscles or extrinsic laryngeal musculature, these muscles were severed bilaterally prior to the final assessment.

Adductor function was assessed on tactile stimulation of the right-sided supraglottic mucosa, resulting in reflex glottic closure in the normal situation. Reflex adduction was evaluated in the two groups in which the vocalis muscle was surgically reinnervated (group 1 and group 3).

Electrical stimulation of the each nerve was performed proximal to the anastomosis, after surgery, while mobility and evoked activity were recorded with videolaryngoscopy and EMG. Following this the animals were killed.

Evaluation of laryngeal mobility

Mobility observed by videolaryngoscopy of the reinnervated right hemilarynx was compared with the normal left hemilarynx. Abduction was scored as *good* if abduction on inspiration occurred almost synchronous with and equal to abduction of the left side; *adequate* if abduction on inspiration was more than half the abduction of the left side; *limited* if abduction on inspiration was less than half; or *poor* if no effective or only very slight abduction on inspiration was observed. Adduction was

cored as *normal* if right hemilarynx adduction was equal to the left hemilarynx; *ecreased* if right-sided adduction was less than the left side; *immobile* if the right emilarynx was immobile and *paradoxical* if right-sided abduction occurred during eft-sided adduction.

EMG evaluation

Electromyography was performed by means of transorally introduced hooked vire electrodes in the left and right PCA muscles, and in the left and right vocalis nuscles (pars medialis of the thyroarytenoid muscle). An electromyograph type MS6 Medelec, Old Walking, UK) was used. Respiratory monitoring was performed imultaneously, using an impedance plethysmograph, to register chest movement.

Results

Laryngeal function 10 weeks after reinnervation surgery

Preoperatively, normal laryngeal mobility and normal respiratory-dependant EMG activity of the PCA muscles and an occasional single motor unit potential in the ocalis muscles was found in all 30 cats. Immediately after the surgical reinnervation ideolaryngoscopy revealed immobility of the right hemilarynx and EMG showed bsence of activity in the right vocalis and PCA muscles. All 10 cats in group 1 could e evaluated during quiet respiration and reflex adduction. During respiratory distress nly 9 cats could be evaluated, as in 1 cat the right RLN was iatrogenically damaged uring the performance of the tracheotomy.

Nine cats could be evaluated in group 2, the remaining cat dying of ostoperative hemorrhage. In group 3, 9 cats were evaluated while 1 cat was excluded ue to iatrogenic damage to the right RLN prior to performing the PN-RLN

Table 7.1 Reflex adduction 10 weeks after reinnervation surgery

Adduction mobility	group 1	group 3
normal	0	0
decreased	3	2
immobile	0	7
paradoxical	7	0

eflex adduction indicates adduction evoked by tactile stimulation of supraglottic mucosa; group 1, anastomosis of the recurrent laryngeal nerve; group 3, selective adductor reinnervation with the nsa cervicalis and selective abductor reinnervation with the phrenic nerve.

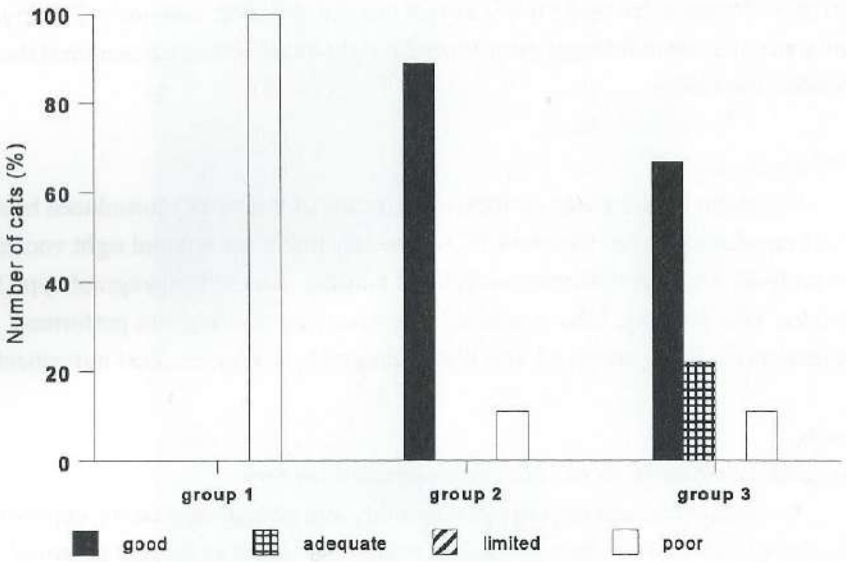


Figure 7.2 *Graph showing abductor function results during quiet respiration, 10 weeks after reinnervation surgery. Group 1: reanastomosis of recurrent laryngeal nerve (n=10); group 2: abductor reinnervation with the phrenic nerve (n=9); group 3: adductor and abductor reinnervation with the ansa cervicalis and PN respectively (n=9).*

anastomosis, precluding successful anastomosis. Results of laryngeal abductor function seen by videolaryngoscopy in each group during quiet breathing are summarized in Figure 7.2. Function during respiratory distress is shown in Figure 7.3. Reflex glottic adduction results are depicted in Table 7.1.

Examples of EMG patterns in the right vocalis and right and left PCA muscles 10 weeks following surgical reinnervation during quiet respiration are shown for group 1 (Figure 7.4), group 2 (Figure 7.5) and group 3 (Figure 7.6). A phasic inspiratory pattern was seen in the normal PCA muscle and in the normal vocalis muscle only sporadic activity in rest and a burst of activity during reflex glottic closure were recorded.

An example of the EMG recordings made on tactile stimulation of the right sided supraglottic mucosa in group 1 is shown in Figure 7.7. Paradoxical activity was observed in the PCA muscle indicating synkinesis due to aberrant regeneration of adductor fibers.

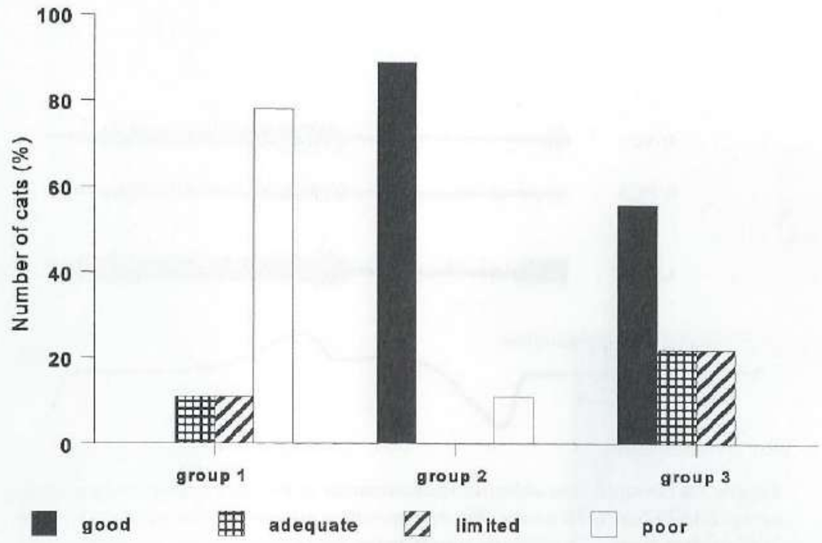


Figure 7.3 *Graph showing abductor function results during respiratory distress, 10 weeks after reinnervation surgery. Group 1: reanastomosis of recurrent laryngeal nerve (n=9); group 2: abductor reinnervation with the phrenic nerve (n=9); group 3: adductor and abductor reinnervation with the ansa cervicalis and PN respectively (n=9).*

Electrical Stimulation

Electrical stimulation was performed proximal to the anastomosis in 9 of the 10 cats in group 1. In all 9 cats laryngeal response to stimulation was observed with videolaryngoscopy and EMG. In the remaining case iatrogenic damage to the RLN occurred during performance of the tracheotomy, precluding electrical stimulation.

In group 2 electrical stimulation of the PN proximal to the anastomosis resulted in abduction of the right vocal fold and an evoked EMG response in the right PCA muscle in 8 of the 9 group 2 cats. In the remaining cat in this latter group the anastomosis was found to have lost continuity. Electrical stimulation of the PN showed abduction of the right vocal fold in all 9 cats in group 3 and an EMG activity response was recorded simultaneously in the right PCA muscle. Electrical stimulation of the right ansa cervicalis sternohyoid branch showed adduction of the vocal fold in all 9 cats, while an EMG response activity was recorded simultaneously in the right vocalis muscle of all these cats.

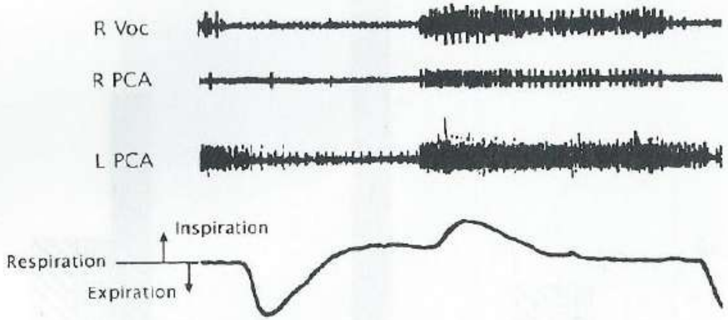


Figure 7.4 *Group 1, non-selective reanastomosis of the right recurrent laryngeal nerve. EMG (2cm/s) 10 weeks after reinnervation surgery during quiet respiration. Note evident signs of synkinesis: simultaneous inspiratory activity in right vocalis (R Voc) and right posterior cricoarytenoid muscles (R PCA); normal inspiratory pattern in left PCA muscle (L PCA). Simultaneous videolaryngoscopy (not shown) demonstrated a virtually immobile right vocal fold with a trembling movement of the arytenoid during inspiration.*

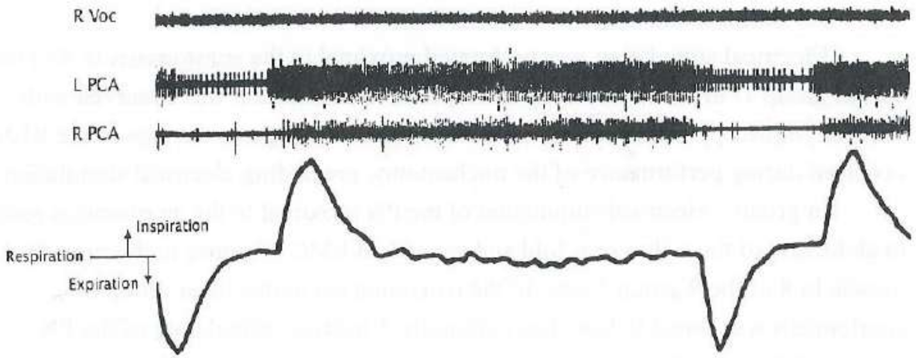


Figure 7.5 *Group 2, selective abductor reinnervation with the phrenic nerve. EMG (2cm/s) 10 weeks after reinnervation surgery during quiet respiration. No EMG activity in the right vocalis muscle (R Voc) and a typical phrenic nerve inspiratory pattern in the right PCA muscle (R PCA); normal inspiratory pattern in left PCA muscle (L PCA). Videolaryngoscopy (not shown) demonstrated abduction on inspiration.*

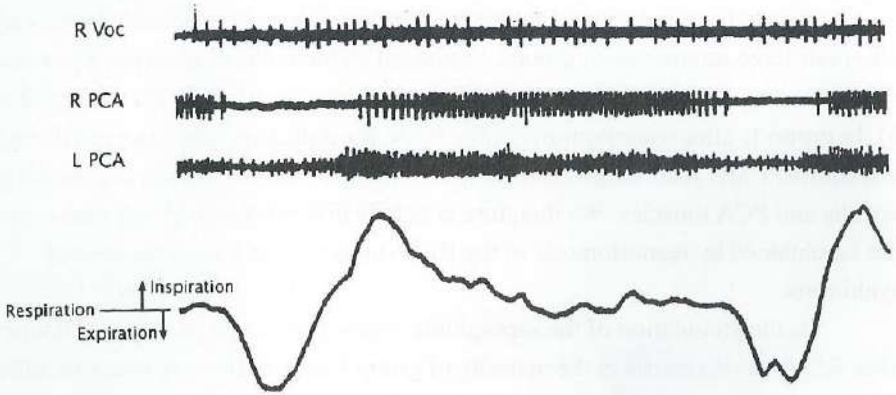


Figure 7.6 *Group 3, selective abductor and adductor reinnervation with the phrenic nerve and ansa cervicalis. EMG (2cm/s) 10 weeks after reinnervation surgery during quiet respiration. Single motor unit activity is observed in the right vocalis muscle (R Voc) and a typical phrenic nerve inspiratory pattern in the right PCA muscle (R PCA); normal inspiratory activity in the left PCA (L PCA). Videolaryngoscopy (not shown) demonstrated abduction on inspiration.*

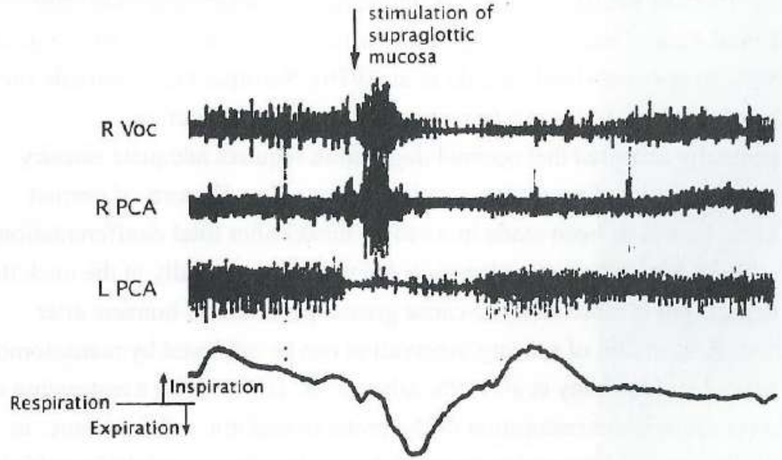


Figure 7.7 *Group 1, non-selective reanastomosis of the right RLN. EMG (2cm/s) 10 weeks after reinnervation surgery during reflex adduction following tactile stimulation of the supraglottic mucosa. Note synkinetic activity in the right vocalis muscle (R Voc) and the right PCA muscle (R PCA). In the left PCA muscle (L PCA) activity is inhibited during the reflex adduction.*

Discussion

Our study demonstrates that laryngeal reinnervation was feasible in the cats in all of our three reinnervation groups. Laryngeal abduction during inspiration was only obtained using selective laryngeal abductor reinnervation with the PN (groups 2 and 3). In group 1, after reanastomosis of the RLN, no abduction was observed during inspiration. EMG recordings demonstrated synkinetic activity during inspiration in vocalis and PCA muscles. We therefore conclude that, a functional restoration could not be obtained by reanastomosis of the RLN (in our group 1 cats) because of synkinesis.

Tactile stimulation of the supraglottic mucosa produced paradoxical abduction after RLN reanastomosis in the majority of group 1 cats. Following selective adductor reinnervation with the ansa cervicalis (group 3) no restoration of reflex adduction was achieved, but no paradoxical abduction was observed either. This was to be expected as the ansa cervicalis does not form a part of the reflex glottic closure arc. This protective reflex is triggered by stimulation of tactile receptors in the glottic and supraglottic mucosa. The stimulus passes along the sensory fibers of the internal branch of the SLN via the nodose ganglion to the nucleus solitarius (the nucleus of the SLN in the brainstem). There is a polysynaptic connection to the nucleus ambiguus and the motor fibers of the RLN, evoking contraction of the laryngeal adductor muscles and inhibition of the phasic inspiratory action potentials in the PCA muscles (Kirchner 1991, Isogai et al. 1991, Sasaki et al. 1976). No other nerve suitable for transfer is therefore likely to be able to restore reflex glottic adduction.

It is generally accepted that optimal deglutition requires adequate sensory innervation and a functional protective glottic closure reflex. Reports of normal deglutition have, however, been made in a canine model after total deafferentation (Berke et al. 1993). Since the canine larynx is located more cranially in the neck than in humans, deglutition is more likely to cause greater problems in humans after deafferentation. Restoration of sensory innervation can be achieved by reanastomosis of the SLN mainstem (Anthony et al. 1995, Silver et al. 1974). Such a restoration of sensory innervation, without restoration of the protective glottic reflex closure, in combination with conditioning and training of the swallowing act might be sufficient to allow swallowing without significant aspiration. Investigation of this is not possible in an animal model.

While our present study has only consisted of a unilateral reinnervation procedure, a method to reinnervate both hemilarynges with only one PN has been described (Baldiserra et al. 1989). The sacrifice of one PN is usually considered to be

ıcceptable (Fackler et al. 1967, Robotham 1979, Easton et al. 1983, Kelly 1950), ılthough in some cases of pre-existing COPD disorders, additional respiratory listurbances might be expected.

In the present study phonation was not investigated. As was mentioned earlier his function appears to have less priority than the other two laryngeal functions as ›atients are often able to phonate reasonably well despite bilateral laryngeal paralyses. Selective adductor reinnervation with the ansa cervicalis did not bring about active ıdduction movement but did reinnervate the muscle group. The resulting muscle tonus s considered to be advantageous for phonatory function.

In conclusion, separate selective reinnervation of laryngeal abductor and ıdductor muscle groups with phrenic and ansa cervicalis nerve transfers, respectively, ıave proved a good option to restore laryngeal motor function in cats. To achieve ›hysiological deglutition following laryngeal motor and sensory reinnervation clearly equires more research before laryngeal transplantation can be considered feasible in ıumans. Once this has been achieved, the gain, risks and costs of transplantation must ›utweigh those of total laryngectomy with optimal speech rehabilitation. Clearly the quality of post-laryngectomy rehabilitation has advanced considerably during the past ears and is no longer comparable to the situation two decades ago when Kluyskens 1972) performed the first laryngeal transplant clinically.

Chapter 8

Effect of Org 2766 on Laryngeal Reinnervation in Cats, A Functional and Histological Analysis

Julie T van Lith-Bijl (MD), Robin J Stolk,
Jeroen ADM Tonnaer (PhD), Cees Groenhout (MD, PhD),
Pierre NM Konings (PhD), Geert JPT Schuijers,
Hilde Tobi, Hans F Mahieu (MD,PhD).

(submitted)

Abstract

Objective To determine the effect of the Adrenocorticotrophin (ACTH) analogue Org 2766 on laryngeal reinnervation in four laryngeal reinnervation conditions: spontaneous reinnervation after crush lesion to the recurrent laryngeal nerve (RLN), reinnervation after transection and neurorrhaphy, selective laryngeal reinnervation with an ansa hypoglossi and phrenic nerve transfer; and a phrenic nerve transfer after delayed reconstruction of the transected RLN.

Design Double-blind placebo controlled randomized trial. Study parameters were: (1) first signs of reinnervation as observed with electromyography; (2) first signs of restoration of laryngeal mobility, (3) assessment of degree of laryngeal abductor function recovery after 10 weeks; and (4) histological analysis of the regenerated nerve in terms of axon density, axon diameter and myelin sheath thickness. These parameters were studied in cats after the four different laryngeal reinnervation conditions.

Results No statistically significant difference was found between Org 2766 treated group and placebo group concerning any of the study parameters, but a tendency toward regeneration of a larger number of thinner axons was disclosed in the histological analysis. This difference however, was not reflected in the functional recovery.

Conclusions Although no significant neurotrophic effect of Org 2766 was found in the four laryngeal reinnervation conditions examined in the cat model, the tendency toward formation of a larger number of thinner regenerated axons supports earlier findings that Org 2766 stimulates axon sprouting.

Introduction

Finely graded control of muscle contraction depends on a large number of innervating motoneurons and small motor units. These factors characterize the laryngeal musculature whose fine and coordinated activity are essential for proper functioning of respiration, swallowing and phonation at the cross roads of airway and digestive tract.

The motoneurons involved in inspiratory abduction and phonatory adduction or reflex glottic closure of the larynx are all located in the recurrent laryngeal nerve (RLN). Reinnervation of the larynx after RLN injury has been the subject of many studies, as recovery of the laryngeal function proved inconsistent and usually insufficient. Studies involving reanastomosis after transection of the nerve showed misdirection of the reinnervating axons to be a major factor in laryngeal function impairment (Chapter 2, Tashiro 1972, Boles et al. 1969, Shimazaki 1957) . Alternatives have been sought to overcome the problems of misdirected reinnervation by application of selective reinnervation procedures with foreign nerve transfers (Chapter 3 & 4, Crumley 1982, 1984, Baldiserra et al. 1986, Marie et al. 1989).

The importance of the number of reinnervating axons and formation of small motor units for the coordination of the reinnervated larynx, however, has received little attention. After injury and regeneration, substantial loss of axons is usually encountered. The smaller number of reinnervating axons usually results in larger motor units, ie, one axon serves a larger number of muscle fibers in the reinnervated muscle, than before denervation.

Theoretically, the higher the number of reinnervating axons the smaller the motorunits formed, and consequently more finely graded control of muscle contraction of the reinnervated muscle. Stimulation of the process of axonsprouting during nerve regeneration could enhance the number of regenerating axons and the formation of small motor units. Nerve regeneration and axonsprouting in rats have successfully been stimulated in such a way by the neurotrophic effects of adrenocorticotrophin (ACTH) and α-melanocyte stimulating hormone (α-MSH) derived peptides (De Koning et al. 1987, Strand et al. 1980, Van der Zee et al. 1989).

The neurotrophic properties of $ACTH_{(4-9)}$ analogue Org 2766 (Met(O_2)-Glu-His-Phe-D-Lys-Phe) have been studied most extensively in the rat sciatic nerve crush model. Functional, histological and immunohistochemical studies have revealed that regeneration of the nerve can be enhanced by peptide treatment in this model probably by stimulation of axon sprouting (Strand et al. 1980, Bijlsma et al.1981, Dekker et al. 1987)

In cats the possibility of stimulating laryngeal reinnervation with Org 2766 has been investigated after selective abductor reinnervation with a phrenic nerve graft (Chapter 3). A tendency toward facilitation of axonsprouting by Org 2766 was found: 26% more regenerating axons in the Org 2766-treated group than in the placebo group. These results are comparable to findings in the rat sciatic nerve crush model (32% more axons) (Tonnaer et al. 1992).

In the present study laryngeal reinnervation with Org 2766 was investigated in the cat model in four reinnervation conditions. These conditions included reinnervation following:

(1) RLN crush injury; (2) transection and reanastomosis of the RLN; (3) selective reinnervation of the laryngeal abductor and adductor muscle groups with a phrenic nerve and ansa cervicalis nerve transfer respectively; and (4) selective abductor

reinnervation with a phrenic nerve transfer 9 months after transection of the RLN.

Materials and Methods

Surgical method

Fifty-two female cats (aged: 6 months; weight range: 2200g-2800g) were anesthetized with ketamine chloride (20mg/kg intramuscularly) and xylazine hydrochloride (0.5mg/kg subcutaneously), allowing spontaneous respiration. Anesthesia was maintained by additional ketamine chloride (10mg/kg intramuscularly) every 10-20minutes.

A midline incision of the neck was performed. Pretracheal muscles were separated in the midline and the upper part of the trachea was exposed. The right RLN was then identified.

In 20 cats a "crush" injury of the right RLN was performed. At a distance of 2.0cm from the cricothyroid joint, the nerve was crushed for 30 seconds with hemostatic forceps with grooved jaws (Halstead No. 02.401.12), by closing the forceps for three clicks (Tonnaer et al. 1992). Immediately after crushing, the distal point of the crush was marked with a suture in the perineurium.

In 10 cats the right RLN was transected at a distance of 2.0cm from the cricothyroid joint. The ends were then reapproximated and reanastomosed with a 10-0 microsuture (Ethilon, Ethicon Inc, Somerville NJ) and fibrin glue (Tissucol, Austrian Institute for Haemoderivates, Vienna, Austria). An extra suture was placed adjacent to the injury site to facilitate identification at a later stage.

In 10 cats a selective laryngeal reinnervation procedure was performed. After transecting the right RLN, the right ansa cervicalis was identified and followed to its sternohyoid branch. The sternohyoid branch was transected near to the sternohyoid muscle. The proximal stump was then transferred to be anastomosed to the adductor branch of the RLN. A small posterior part of the thyroid cartilage was resected to expose the adductor and abductor RLN nerve branches. The abductor branch was preserved and the adductor branch was transected, the proximal adductor stump was buried in the right posterior cricoarytenoid (PCA) muscle and fixed with fibrin glue. The distal adductor stump was anastomosed to the proximal stump of the sternohyoid branch of the ansa cervicalis with a 10-0 microsuture and fibrin glue. To exclude any influence of the ansa galeni (Diamond et al. 1992), although unlikely because of its allegedly sensory nature, it was also transected and its proximal stump buried in the right PCA muscle. The 2 roots of the right phrenic nerve (PN) were identified. In

contrast to the situation in humans, the PN in the cat has two clearly distinguishable roots. The uppermost PN root was resected just before it joined the other root. The right RLN was transected about 2.0cm below the larynx and the distal RLN stump was anastomosed to the proximal PN stump using a 10-0 microsuture and fibrin glue. Thus (Figure 4.1) all reinnervating PN axons were directed towards the PCA muscle, even those that followed the path of the adductor branch or the ansa galeni.

In 12 cats the RLN was transected at 2.0cm from the cricothyroid joint, and a length of approximately 4-5cm was removed from the proximal stump. Nine months later the distal stump was identified cut near its end and anastomosed to the phrenic nerve. A small posterior part of the thyroid cartilage was resected to expose the adductor and abductor RLN branches. The abductor branch was preserved, the adductor branch and ansa galeni were transected, the proximal stumps were buried in the PCA muscle and fixed with fibrin glue.

Org 2766

The cats in each reinnervation procedure group were randomly divided into group A and group B, to receive solution A or B, respectively. At the time of study it was unknown to the investigators which solution contained Org 2766 or the vehicle (0.9% saline) as placebo. Starting immediately postoperatively each cat was given 0.01ml/kg subcutaneously of solution A or solution B, every 48 hours during 28 days. Org 2766-treated cats received the compound at a dose of 25μg/kg/48 hours.

Evaluation of reinnervation and laryngeal mobility

Using the same anesthesia that was used for the surgical procedure, the cats were evaluated weekly. Using videolaryngoscopy laryngeal mobility was observed. The mobility of the reinnervated right hemilarynx was compared with the normal left hemilarynx. Using electromyography (EMG) reinnervation was evaluated. EMG was performed by means of transorally introduced hooked wire electrodes and an electromyograph type MS6 (Medelec, Old Walking, England). EMG recordings were performed in the left and right posterior cricoarytenoid (PCA) muscle, and in the left and right vocalis muscles (pars medialis of the thyroarytenoid muscle). Respiratory monitoring was performed simultaneously, using a custom-made impedance plethysmograph, registering chest movement.

Study parameters

(1) *First electromyographical signs of reinnervation*

EMG was performed before and immediately after the surgical procedure, and thereafter weekly. The time at which first signs of reinnervation were observed in the right vocalis and right PCA muscles was recorded.

(2) *First signs of restoration of mobility*

Videolaryngoscopy was performed before and immediately after the surgical procedure, and thereafter weekly. The time at which a first sign of mobility of the right hemilarynx was observed was recorded.

(3) *Final assessment of laryngeal abductor function*

Ten weeks after the surgical procedure, laryngeal abductor function was evaluated using videolaryngoscopy, during normal respiration. To eliminate any influence of the cricothyroid muscles or extrinsic laryngeal musculature, these muscles were severed bilaterally prior to the final assessment. Abduction was scored as *good* if abduction on inspiration occurred almost synchronous with and equal in degree of abduction to the abduction of the left side; *adequate* if abduction on inspiration was more than half the abduction of the left side; *limited* if abduction on inspiration was less than half or half the abduction of the left side; and *poor* if no effective or very slight abduction on inspiration was observed.

(4) *Final electromyographical assessment and response to electrical stimulation*

Ten weeks after the surgical procedure, reinnervation was evaluated by performing EMG of the left and right vocalis muscles and the left and right PCA muscles. The crush injury site or anastomosis was then identified and electrically stimulated (1mAmp, 50 Hz) proximal to this site. The laryngeal response was observed by videolaryngoscopy and electromyography.

(5) *Histological evaluation*

After 10 weeks, the right RLN was fixed in situ in the anesthetized animal using cacodylate-gluteraldehyde solution and was resected. After postfixation in the same solution, the tissue was embedded in epon and 1µm thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5cm distal to the anastomosis. By computerized screening of a 500-µm-wide band across the widest diameter, the axon density, axon diameter and thickness of the myelin sheath were estimated.

Statistical analysis

The time taken for first signs of laryngeal mobility and reinnervation to appear was evaluated using the Mantel Cox log rank test, α was corrected for multiple comparisons and taken at 0.005. The effect of administration of Org 2766 or placebo on these parameters was also evaluated with the Mantel-Cox log rank test.

The restoration of abductor function was analyzed using the Mann-Whitney U-test to compare outcomes of all reinnervation conditions and the effect of Org 2766 administration per reinnervation condition. Again α was corrected for multiple comparisons and taken at 0.005.

To compare the effect of Org 2766 and placebo on the histological parameters: axon density, axon diameter and myelin thickness an analysis of variance was used.

Results

Two cats could not be evaluated, 1 cat in the selective reinnervation group, in which an inconsistency in the surgical procedure precluded successful reinnervation and 1 cat in the delayed reinnervation group which died during the delay period.

Study parameters

The mean times at which (1) *first electromyographical signs of reinnervation* and (2) *first signs of restoration of mobility* were observed are demonstrated in Table 8.1. The mean time at which first signs of reinnervation (EMG activity) appeared in the PCA and in the vocalis muscles as well as the mean time at which a first sign of mobility restoration was seen using videolaryngoscopy was significantly earlier following crush injury than following the surgical reinnervation procedures (Mantel-Cox, $p<0.005$). The time at which first signs of reinnervation were seen in the PCA muscle after the different types of surgical reinnervation procedures (RLN-reanastomosis, selective abductor reinnervation with PN and delayed reinnervation) did not differ ($p>0.05$). Org 2766 appeared to have no significant effect on the recovery periods concerning both EMG and laryngeal mobility parameters when compared with the placebo-treated animals in any of the four reinnervation conditions. The p values obtained with the Mantel-Cox (log-rank) test are shown in Table 8.1.

3)Results of the final assessment of abductor function for the four reinnervation groups and Org 2766 / placebo are given in Table 8.2. Org 2766 had no significant effect on the abduction mobility in any of the reinnervation groups although a strong tendency toward a better abductor function in the Org 2766 treated group was found in the delayed reinnervation group (p=0.07). The best abduction mobility resulted after crush injury, the poorest after RLN reanastomosis (p<0.0001 Mann-Whitney). Good abduction mobility was achieved using the PN-RLN and AC-add anastomoses and did not differ significantly from the results after crush injury (p=0.41). The abduction restoration after delayed reinnervation was clearly poorer than after selective reinnervation in the acute stage and better than after RLN reanastomosis but did not differ significantly from either of the two groups (p=0.37 and 0.18, respectively).

Table 8.1 Mean time of first sign of reinnervation after recurrent laryngeal nerve crush injury or surgical reinnervation

Surgical procedure		Number of cats	Mobility (weeks)	EMG R Voc (weeks)	EMG R PCA (weeks)
CRUSH injury	**Org 2766**	10	2.7[0.2]	2.2[0.1]	2.8[0.1]
	Placebo	10	2.3[0.1]	2.6[0.2]	3.1[0.2]
	p(Mantel-Cox)		*0.14*	*0.08*	*0.18*
RLN-RLN reanastomosis	**Org 2766**	5	3.8[0.2]	3.6[0.5]	4.2[0.4]
	PLacebo	5	4.0[0.5]	4.2[0.5]	3.4[0.4]
	p(Mantel-Cox)		*0.94*	*0.4*	*0.26*
PN-RLN and AC-add anastomosis	**Org 2766**	5	6.2[1.1]	5.0[0.5]	5.0[0.6]
	Placebo	4	4.3[0.7]	4.3[0.3]	4.0[0.6]
	p(Mantel-Cox)		*0.19*	*0.19*	*0.35*
PN-RLN after 9 months delay	**Org 2766**	6	6.0[0.9]	9.0[0.5]	5.0[1.0]
	Placebo	5	7.2[0.8]	8.8[0.8]	4.4(0.8]
	p(Mantel-Cox)		*0.41*	*0.96*	*0.68*

Table 8.2 Laryngeal abductor function 10 weeks after recurrent laryngeal nerve crush injury or surgical reinnervation

Surgical procedure		Number of cats	Mobility of right hemilarynx				*p (Mann-Whitney)*
			good	adequate	limited	poor	
CRUSH injury	**Org 2766**	10	9	1	0	0	*0.25*
	Placebo	10	7	2	1	0	
RLN-RLN reanastomosis	**Org 2766**	5	0	0	0	5	*1*
	Placebo	5	0	0	0	5	
PN-RLN and AC-add anastomosis	**Org 2766**	5	3	1	0	1	*0.56*
	Placebo	4	3	1	0	0	
PN-RLN after 9 months delay	**Org 2766**	6	3	1	0	2	*0.07*
	Placebo	5	0	0	1	4	

(4) *Final electromyographical assessment and response to electrical stimulation*

Nerve regeneration and subsequent reinnervation results as evaluated by EMG and electrical stimulation, are shown in Table 8.3. In 1 cat avulsion of the nerve anastomosis precluded reinnervation in the delayed reinnervation group. In all other cats successful regeneration and reinnervation had taken place according to the EMG results and electrical stimulation. Simultaneous activity was observed in the right vocalis and PCA muscles after RLN-reanastomosis in the 5 cats treated with Org 2766 and in the 5 cats treated with placebo.

(5) *Histological analysis*

Three nerve specimens were lost to evaluation due to inconsistencies in the fixation procedure although successful regeneration and reinnervation had taken place according to the electrical stimulation results and EMG. The specimen from the cat with avulsion of the nerve-anastomosis histological examination was not performed. The overall results are given in Table 8.3. After crush injury a tendency toward a larger number of thicker regenerated axons was found with thicker myelin sheaths than in the other three reinnervation conditions. A consistent tendency toward formation of thinner regenerating axons (axon diameter) was observed in the Org 2766-treated group compared with placebo but this was not statistically significant in any of the groups (analysis of variance p=0.18).

Table 8.3 Reinnervation results 10 weeks after recurrent laryngeal nerve crush injury or surgical reinnervation

Surgical procedure		Number of cats	Response on electrical stimulation at 10 weeks	EMG proof of reinnervation at 10 weeks	Number of macroscopic avulsions	Number of specimens analyzed	Median axon count (number of axons per mm²)	Median axon diameter (µm)	Median myelin thickness (µm)
CRUSH injury	Org 2766	10	10	10	0	9	4690 *(CI:3070-6309)*	2.78 *(CI:2.36-3.21)*	7.30 *(CI:6.48-8.11)*
	Placebo	10	9	10	0	10	4395 *(CI:2859-5931)*	2.90 *(CI:2.50-3.30)*	7.91 *(CI:7.13-8.69)*
RLN-RLN reanastomosis	Org 2766	5	5	5	0	5	4909 *(CI:2737-7082)*	2.21 *(CI:1.64-2.78)*	7.32 *(CI:6.22-8.42)*
	Placebo	5	4	5	0	4	3383 *(CI:954-5812)*	2.43 *(CI:1.79-3.07)*	6.64 *(CI:5.41-7.87)*
PN-RLN and AC-add anastomosis	Org 2766	5	5	5	0	4	2081 *(CI:347-4510)*	1.88 *(CI:1.24-2.51)*	6.42 *(CI:5.19-7.65)*
	Placebo	4	4	4	0	4	4113 *(CI:1685-6542)*	2.06 *(CI:1.42-2.70)*	6.41 *(CI:5.17-7.63)*
PN-RLN after 9 months delay	Org 2766	6	5	5	1	4	4671 *(CI:2242-7099)*	1.82 *(CI:1.18-2.46)*	6.35 *(CI:5.12-7.57)*
	Placebo	5	5	5	0	4	2871 *(CI:442-5300)*	2.53 *(CI:1.89-3.16)*	6.24 *(CI:5.01-7.47)*

Discussion

As the outcomes in terms of restoration of mobility differed between the four reinnervation conditions, we felt the groups could not be combined to determine the effect of Org 2766 treatment compared with placebo. The effect of Org 2766 was therefore considered separately for each reinnervation condition. No differences were found in the mean times at which first signs of recovery of mobility and reinnervation of the vocalis and PCA muscles were observed, in any of the reinnervation conditions. These results do not further support the findings in our earlier study concerning the effect of Org 2766 treatment in selective abductor reinnervation with the phrenic nerve in cats, where a tendency toward a shorter mean reinnervation time was observed in the Org 2766 group compared with the Placebo-treated group.

The recovery of abductor function 10 weeks after injury or surgical reinnervation appeared uneffected by Org 2766 treatment. Only in the delayed reinnervation condition a strong tendency toward a better abductor function restoration was seen in the Org 2766-treated cats ($p=0.07$). Abductor function results in this group were found also to be influenced by a reduced mobility of the cricoarytenoid joint (Chapter 5). An aspect associated with prolonged laryngeal immobility in animals (Langnickel 1973). Correction for the factor of reduced mobility of the cricoarytenoid joint was not feasible for these small numbers.

Abductor function restoration was found to be devastatingly influenced by synkinesis, in the cats in which this was encountered. The occurrence of synkinesis was similar in the Org 2766- and placebo-treated cats. In the reinnervation conditions in which synkinesis could be largely prevented, abduction on inspiration was good leaving little room for improvement. The type of reinnervation procedure therefore proves to have more influence on the functional outcome of laryngeal reinnervation than administration of Org 2766.

A consistent tendency was observed in the histological analysis towards formation of thinner axons in the Org 2766-treated group and, with the exception of selective abductor and adductor reinnervation with the phrenic nerve and ansa cervicalis, a tendency toward formation of a larger number of axons. This finding is in accordance with earlier observed results in the crush injury in the sciatic nerve in rats (Tonnaer et al. 1992) and supports the theory that Org 2766 enhances reinnervation by stimulation of sprouting of regenerating axons. This difference, however, was not reflected by an improvement of the functional results. In laryngeal reinnervation the problem of synkinesis remains the most important adverse aspect which has to be avoided to achieve good functional results. What was originally considered to be an interesting model to investigate the influence of Org 2766 on functional recovery, because of the small motor units in the larynx, has proved to be unsuitable for this purpose.

Chapter 9

Summary and Conclusions

In **Chapter 1, the introduction,** the laryngeal functions, anatomy and physiology have been described. The larynx has 3 major functions. It serves to protect the airway from aspiration, to maintain optimal airway and its vocal folds generate vocal sound. Proper functioning of the larynx is a result of coordinated activity of the abductor and adductor muscles, opening and closing the vocal folds, respectively. The abductor and adductor nerve fibers are intertwined throughout the length of the recurrent laryngeal nerve (RLN) until they divide into their respective branches close to the target muscles.

Injury to the RLN can result in impairment of all three laryngeal functions. The RLN is capable of regeneration but laryngeal functions usually remain impaired. This impairment is caused largely by the occurrence of laryngeal synkinesis. A review of surgical laryngeal reinnervation techniques is given. Considering the various reinnervation techniques and results, selective reinnervation seems to be the most promising option. Hereby the opposing adductor and abductor muscle groups are separately reinnervated with foreign nerves and synkinesis may theoretically be avoided. We investigated reinnervation in a cat model, with the following aims:

- to investigate restoration of laryngeal function after different types of nerve injury
- to confirm the feasibility of selective laryngeal reinnervation with respect to abductor and adductor function
- to determine the influence of delay of selective laryngeal reinnervation on the functional outcome
- to determine the influence of nerve regeneration enhancement by administration of Org 2766 on functional outcome
- to develop a management protocol for laryngeal nerve injury on the basis of animal experiments and clinical experience.

In **Chapter 2** restoration of laryngeal function was investigated after crush injury to the RLN in 20 cats and compared with the laryngeal function results after complete transection of the nerve followed by immediate reanastomosis in 10 cats. The standardized crush injury was designed to damage the nerve axons but preserve the endoneurial tubes surrounding the individual axons. Transecting the nerve resulted in discontinuity of both the axons as well as the endoneurial tubes. Good recovery of function occurred after the crush injury whereas poor results with limited abductor mobility or immobility resulted after reanastomosis of the RLN. The poor results after RLN-reanastomosis were caused by the well-known phenomenon of misdirection of

regenerating axons resulting in laryngeal synkinesis. The regenerating axons after crush injury were directed to their original muscles guided by the intact endoneurial tubes whereas the regenerating fibers after transecting the nerve, grew at random, partly into inappropriate tubes to eventually reach and reinnervate the wrong target muscle. The resulting synkinetic activity of opposing adductor and abductor muscle groups resulted in an uncoordinated ineffective laryngeal function.

It was concluded that normal restoration of abductor function will occur after crush injury, whereas no effective abductor function restoration occurs after reanastomosis of the RLN, because of misdirection of regenerating axons resulting in inappropriate reinnervation and laryngeal synkinesis.

In **Chapter 3** selective abductor reinnervation was studied in 10 cats using a phrenic nerve transfer. Reinnervation of the PCA muscle occurred and resulted in excellent abduction mobility synchronous with inspiration. The phrenic nerve has an inspiratory activity pattern which is similar to the pattern observed in the abductor axons in the RLN and thus serves as an excellent substitute to reinnervate the abductor musculature. Using a selective reinnervation procedure to reinnervate only the abductor prevented the development of laryngeal synkinesis and using the phrenic nerve, good restoration of abductor function can be achieved.

Concurrently, in this study, double-blind administration of the neurotrophic neuropeptide Org 2766 or placebo was performed. Although no significant effect could be detected, a strong tendency toward earlier reinnervation was found in the Org 2766-treated group, and histological analysis also demonstrated a tendency toward formation of a larger number of thinner axons in the regenerated nerves in the Org 2766-treated group.

In **Chapter 4** selective adductor muscle reinnervation was performed in combination with selective reinnervation of the abductor muscle with the phrenic nerve in10 cats. For recovery of adductor muscle function the donor nerve should have a similar activity pattern to the adductor fibers with enhanced activity during phonation and protective reflex closure. As no optimally suitable nerve is available for this purpose we chose the ansa cervicalis which has a very weak activity pattern during normal breathing. The ansa cervicalis innervates the strap muscles which have an accessory respiratory function, and an inspiratory activity pattern only during respiratory distress. Reinnervation of the adductor muscles with the ansa cervicalis resulted in a recovery of muscle tonus during normal breathing. During respiratory distress, simultaneous inspiratory activity occurred in the adductors as well as the abductors. The activity generated by the phrenic nerve, by far outweighed that of the ansa cervicalis and the synkinetic activity resulted only in a slight compromise of the abductor function.

It is concluded that both the abductor and adductor muscle groups can be simultaneously reinnervated to obtain good abductor function, as well as restoration of adductor mobility. Active reflex glottic closure, using the ansa cervicalis to substitute the adductor, was not achieved.

Chapter 5. Since usually in clinical practice quite some time elapses between the time of injury and the time of treatment, research on the effect of this delay period is essential, if reinnervation is contemplated in humans. Selective reinnervation using a phrenic nerve transfer was performed 9 months after transecting the RLN in 11 cats. After the 9 month delay period signs of spontaneous subclinical reinnervation had occurred in 7 of the 11 cats. These signs were abolished after retransecting the RLN in 4 cats and in 3 a very minor activity pattern persisted. Reinnervation of the PCA muscle using selective abductor reinnervation with the phrenic nerve, did occur, but recovery of abductor function was clearly less successful than after selective abductor reinnervation in the acute phase. The main cause of this impairment seemed to be a reduced mobility of the cricoarytenoid joint, a phenomenon which has been described in rabbits after RLN paralysis, but is relatively rare in humans. The persistence of subclinical reinnervation in the PCA muscle at the time of reinnervation did not have any influence on the reinnervation by the phrenic nerve. In conclusion, the sooner a reinnervation procedure is performed the better, as earlier intervention prevents progression of subclinical reinnervation and fixation of the cricarytenoid joint. Further research should concentrate on development of techniques such as laryngeal EMG, which can accurately predict prognosis at an early stage after RLN paralysis.

Chapter 6. Acute unilateral RLN injury, such as could be encountered after trauma or surgical procedures in the neck or thorax will usually give rise to phonatory problems and to a lesser extent to aspiration, but the long term problems are unpredictable in the acute phase. As no clear consensus exists for management of transection of the RLN in the acute phase, adductor function restoration was investigated in a cat model. Adductor function recovery was evaluated after crush injury (20 cats), RLN-reanastomosis (10 cats) and selective adductor reinnervation with the ansa cervicalis and abductor reinnervation with the phrenic nerve (10 cats). As a control the RLN was transected and left unrepaired, and 9 months later the adductor function was also evaluated (11 cats). In all conditions, spontaneous adduction during normal respiration and reflex adduction on mechanical stimulation of the supraglottic mucosa was examined. Crush injury resulted in normal recovery of adductor function. After RLN-reanastomosis, synkinesis and severe impairment of adductor function during normal breathing as well as paradoxical mobility during laryngeal protective reflex occurred. Ansa cervicalis anastomosis to the adductor branch resulted in reinnervation, recovery of muscle tonus, normal spontaneous adductor mobility during normal breathing, but no recovery of reflex adduction. RLN transection without repair showed no recovery of adductor function.

Two patient histories after RLN-reanastomosis in the acute phase, with poor functional outcome, are described. Based on the above described experiments and clinical practice a management algorithm is proposed for treatment of unilateral RLN crush injury or transection in the acute phase.

In conclusion, reanastomosis of the transected RLN is contraindicated. Selective adductor reinnervation can be considered in the acute phase especially as improved phonation may be expected in patients, but laryngeal framework surgery in a later stage is also a good treatment option.

Chapter 7. In laryngeal transplantation, laryngeal reinnervation forms an essential aspect, together with adequate revascularization and immunosuppression, as restoration of laryngeal function depends on appropriate reinnervation. Our experiments in the cat model showed that restoration of a good respiratory function can be achieved. Optimal deglutition requires adequate sensory innervation and a functional protective glottic closure reflex. Successful restoration of sensory reinnervation has been reported (Anthony et al. 1995, Silver et al. 1974) by reanastomosis of the superior laryngeal nerve mainstem. Selective reinnervation of the adductor with the ansa cervicalis as was performed in our study did not, however, bring about adequate restoration of glottic reflex closure. Although restoration of respiratory function is feasible, further investigations are still required before optimal restoration of all laryngeal function may be expected after laryngeal transplantation.

Chapter 8. The effect of the neurotrophic neuropepetide Org 2766 was tested by integrating a double blind Org 2766/placebo trial in the different groups of cats. Finely graded control of muscle contraction depends on a large number of innervating motoneurons and small motor units. These factors characterize the laryngeal musculature whose fine and coordinated activity are essential to normal laryngeal function at the crossroads of airway and digestive tract. Org 2766 has been shown to enhance nerve regeneration by stimulation of nerve sprouting and could thus stimulate formation of smaller motor units. Laryngeal function results and reinnervation results with respect to Org 2766 were considered. A consistent tendency toward formation of thinner regenerated axons was found. This finding was in accordance with earlier observed results after crush injury in the sciatic nerve in rats (Tonnaer et al. 1992) and supports the theory that Org 2766 enhances reinnervation by stimulation of sprouting of regenerating axons. These results were, however, not reflected by any detectable improvement in laryngeal function recovery, in comparison to the placebo treated cats. In conclusion, Org 2766 was not found to have any significant effect on reinnervation or function recovery.

Samenvatting

In **Hoofdstuk 1, de introductie,** wordt de anatomie en fysiologie van de larynx beschreven. De larynx heeft 3 belangrijke functies: Bescherming van de ademweg bij het slikken teneinde aspiratie te voorkomen, behoud van een open ademweg en generatie van stemgeluid. Goed functioneren van de larynx is het resultaat van een gecoördineerde activiteit van de abductor en adductor spieren die de stemplooien, respectievelijk, openen en sluiten. De zenuwvezels die deze spieren verzorgen verlopen in een vlechtwerk door de n.recurrens totdat ze hun afzonderlijke takken bereiken, dichtbij de doelspieren. Beschadiging van de n.recurrens kan resulteren in uitval van alle 3 laryngeale functies.

Een literatuur overzicht van chirurgische laryngeale reinnervatie technieken wordt beschreven. Herstel van de laryngeale functies blijft meestal uit ondanks eventuele regeneratie van de n.recurrens. Deze geringe herstelkans wordt grotendeels veroorzaakt door het veelvuldig voorkomen van laryngeale synkinesieën. Van alle reinnervatie technieken lijkt selectieve reinnervatie van de abductor en adductor spieren de beste optie, hiermee zouden laryngeale synkinesieën kunnen worden voorkomen.

In deze studie werd laryngeale reinnervatie onderzocht in een katten model met de volgende doelstellingen:

- Evaluatie van het herstel van de laryngeale functies na verschillende soorten zenuwbeschadiging
- Bevestiging van herstel van abductor- en adductorfunctie met selectieve laryngeale reinnervatie
- Onderzoek naar de mogelijkheid van een uitgestelde selectieve reinnervatie
- Bepaling van het effect van een zenuwregeneratie bevorderend middel (Org 2766) op het functionele herstel
- Ontwikkeling van een behandelingsprotocol voor enkelzijdige beschadiging van de n.recurrens op basis van onze dierexperimentele resultaten en klinische ervaringen.

In **Hoofdstuk 2** worden de resultaten van laryngeaal functieherstel vergeleken na kneuzing van de n.recurrens (20 katten), en na doornemen van de n.recurrens gevolgd door continuïteitsherstel (reanastomose) (10 katten). De gestandaardiseerde kneuzing had tot doel de axonen te beschadigen maar de endoneurale buizen, die de individuele axonen omsluiten, intact te laten. Doornemen van de n.recurrens resulteerde in volledige discontinuïteit van zowel de axonen als de endoneurale buizen. Na zenuwkneuzing trad er over het algemeen een goed herstel op. Na n.recurrens reanastomose bleef herstel van abductie uit. Het uitblijven van herstel van abductie na n.recurrens reanastomose, werd veroorzaakt door het bekende fenomeen van misdirectie van regenerende axonen en laryngeale synkinesieën. Na zenuwkneuzing groeien de regenerende axonen langs de intacte endoneurale buizen naar de juiste doelspier. Na n.recurrens reanastomose kunnen de regenerende vezels gedeeltelijk andere neurale buizen ingroeien en zodoende een onjuiste doelspier bereiken. Laryngeale synkinesieën ontstaan door gelijktijdige activiteit in de tegengestelde spiergroepen en leiden daardoor tot ineffectieve laryngeale functie. Geconcludeerd wordt, dat de abductiefunctie zich na zenuwkneuzing herstelt, maar na doornemen en reanastomose van de n.recurrens blijft herstel uit door misdirectie van regenererende zenuwvezels, onjuiste reinnervatie en laryngeale synkinesieën.

In **Hoofdstuk 3** werd selectieve abductor reinnervatie met de n.phrenicus bestudeerd bij 10 katten. Reinnervatie van de m.posticus, de enige ware abductor, werd verkregen met uitstekende abductie, synchroon met de inademing. De n.phrenicus heeft een inspiratoir activiteitspatroon vergelijkbaar met het activiteitspatroon van de abductor axonen in de n.recurrens en is zodoende een geschikte donorzenuw. Met selectieve reinnervatie van de abductor werden laryngeale synkinesieën voorkomen en het gebruik van de n.phrenicus zorgde voor een goed herstel van abductie.

In **Hoofdstuk 4** wordt selectieve reinnervatie van de adductoren verricht in combinatie met de selectieve reinnervatie van de abductor met de n.phrenicus (10 katten). De donorzenuw voor de adductoren zou bij voorkeur een toename van activiteit moeten hebben bij fonatie en reflectoire adductie (bij slikken of hoesten). Omdat er geen optimale donorzenuw voor de adductoren voorhanden is, werd gekozen voor de ansa cervicalis. De ansa cervicalis innerveert de extrinsieke laryngeale spieren, die als hulpademhalingsspieren fungeren. Deze hebben een basisactiviteit in rust en een inspiratoir activiteitspatroon in ademnood. Reinnervatie van de adductor spieren met de ansa cervicalis zorgde voor een herstel van de spiertonus bij normale ademhaling. Bij ademnood werd een inspiratoir aciviteitspatroon gezien in de m.vocalis gelijktijdig met inspiratoire activiteit in de m.posticus. Deze synkinetische activiteit uitte zich echter maar in een geringe beperking van abductie bij iedere inspiratie. De activiteit in de adductoren werd kennelijk overheerst door de n.phrenicus activiteit in de abductor spier. Geconcludeerd wordt dat de adductor en abductor spieren gelijktijdig door

verschillende donorzenuwen kunnen worden gereinnerveerd. Hiermee treedt een goed herstel van abductorfunctie en enige adductiemobiliteit op. Actieve reflectoire glottissluiting blijft echter uit.
Met deze techniek kan een goede respiratoirefunctie met redelijke fonatie worden bereikt, maar zal waarschijnlijk onvoldoende bescherming geven van de ademweg bij slikken.

Hoofdstuk 5. Indien in een klinische situatie de n.recurrens beschadigd wordt, verstrijkt over het algemeen enige tijd alvorens een behandeling wordt ingesteld. Onderzoek naar het effect van een uitgestelde reinnervatie is daarom essentieel. Selectieve abductor reinnervatie werd verricht 9 maanden na doornemen van de n.recurrens bij 11 katten. Preoperatief was al bij 7 van de 11 katten spontane subklinische reinnervatie opgetreden. Deze spontane reinnervatie verdween na opnieuw doornemen van de n.recurrens bij 4 katten. Bij de andere 3 katten bleef er een zeer zwak activiteitspatroon bestaan.
Chirurgische reinnervatie van de m.posticus werd in alle katten bewerkstelligd door selectieve reinnervatie van de abductor met de n.phrenicus, maar het mobiliteitsherstel van de abductor was duidelijk minder goed dan wanneer deze selectieve reinnervatie van de abductor wordt verricht in de acute fase. De belangrijkste oorzaak bleek een verminderde mobiliteit van het crico-arytenoid gewricht, een fenomeen dat bij konijnen is beschreven na n.recurrens paralyse, maar relatief zeldzaam blijkt bij de mens. Het persisteren van subklinische spieractiviteit na opnieuw doornemen van de n.recurrens bleek geen nadelige invloed te hebben op de reinnervatie met de n.phrenicus.
Er wordt geconcludeerd dat een zo kort mogelijk interval tussen beschadiging van de n.recurrens en reinnervatie poging gunstig is. Hiermee kan progressie van subklinische reinnervatie en fixatie van het crico-arytenoid gewricht worden voorkomen. Verder onderzoek naar een methode om de prognose van herstel na n.recurrens uitval zo spoedig mogelijk en betrouwbaar te kunnen stellen zou moeten worden verricht, op basis van bijvoorbeeld electromyografisch onderzoek.

Hoofdstuk 6. Bij acute enkelzijdige beschadiging van de n.recurrens, hetgeen op kan treden bij trauma of bij chirurgische ingrepen van hals of thorax, treedt uitval van abductie- en adductiefunctie op. Klinisch blijkt de adductieuitval het belangrijkst gezien de fonatie problematiek en in mindere mate het voorkomen van aspiratie. Er is geen consensus over het beleid bij een doorgenomen n.recurrens in de acute fase. De problemen op de langer termijn zijn niet goed te voorspellen in de acute fase.
In deze studie werd herstel van adductiefunctie onderzocht in het kattenmodel. Herstel van de adductiefunctie werd bestudeerd na kneuzing van de n.recurrens (20 katten), na n.recurrens reanastomose (10 katten) en na selectieve reinnervatie van de adductoren met de ansa cervicalis (en reinnervatie van de abductor met de n.phrenicus) (9 katten). Bij een controle groep werd de n.recurrens doorgenomen en niet gereconstrueerd (11 katten), 9 maanden later werd de adductiefunctie geëvalueerd. De spontane adductiemobiliteit bij normale ademhaling en reflectoire adductie gestimuleerd door aanraken van de supraglottische mucosa werden onderzocht. Na zenuwkneuzing trad er een normaal herstel op. Na n.recurrens reanastomose werd door het optreden van synkinesieën vrijwel geen herstel van spontane adductiefunctie gezien en bij supraglottische stimulatie, zelfs paradoxale activiteit. Ansa cervicalis reinnervatie van de adductor resulteerde in herstel van spiertonus en normale spontane adductiemobiliteit bij normale ademhaling, maar geen herstel van reflectoire adductie. Doornemen van de n.recurrens zonder reconstructie toonde geen herstel van adductie.

Twee patiënten casus met een slechte larynxfunctie na n.recurrens reanastomose in de acute fase worden beschreven. Op basis van de beschreven experimentele resultaten en enkele ervaringen uit de kliniek werd een behandelingsprotocol opgesteld voor eenzijdige n.recurrens kneuzing of transectie in de acute fase.
Concluderend, is reanastomose van een doorgenomen n.recurrens gecontraindiceerd. Selectieve adductor reinnervatie zou eventueel in de acute fase kunnen worden overwogen. Bij patiënten zal de selectieve reinnervatie van de adductoren de stem in ieder geval ten goede komen, eventueel kan in een later stadium een stemverbeterende ingreep ook nog kunnen worden overwogen.

Hoofdstuk 7. Laryngeale reinnervatie vormt een essentieel onderdeel van larynx transplantatie. Voor herstel van laryngeale functie is adequate reinnervatie vereist. Uit onze experimenten in het kattenmodel blijkt herstel van de respiratoirefunctie goed mogelijk. Voor een optimaal verlopende slikactie is adequate sensibele innervatie en een normaal functionerende reflectoire adductie noodzakelijk. Sensibele reinnervatie verkregen met reanastomose van de hoofdstam van de n.laryngeus superior is reeds in de literatuur beschreven (Anthony et al. 1995, Silver et al. 1974). Selectieve reinnervatie van de adductoren met de ansa cervicalis, zoals in onze studie, zorgt niet voor een adequate reflectoire adductie. Hoewel herstel van de respiratoirefunctie goed mogelijk is, zal verder onderzoek moeten worden verricht naar voldoende herstel van de 3 belangrijke laryngeale functies na larynx transplantatie.

Hoofdstuk 8. Het effect van de neurotrofe neuropeptide Org 2766 werd onderzocht middels integratie van een dubbelblinde, placebo gecontroleerde trial bij de verschillende groepen katten. Nauwkeurige controle van spier contracties is afhankelijk van een groot aantal innerverende zenuwvezels en kleine motor units. Deze factoren karakteriseren de laryngeale spieren, waarvan de nauwkeurig gecoördineerde activiteit van essentieel belang is voor het functioneren van de larynx op het kruispunt van bovenste luchtweg en spijsweg. Bij Org 2766 is aangetoond dat het sprouting van regenerende axonen stimuleert en zodoende formatie van kleinere motor units kan bevorderen. Laryngeale functie resultaten en reinnervatie uitkomsten met betrekking tot toediening van Org 2766 of placebo worden geëvalueerd. Een consistente tendens tot vorming van dunnere zenuwvezels werd gevonden. Deze resultaten zijn in overeenstemming met de bevindingen van een reeds eerder verricht onderzoek met Org 2766 na kneuzing van de n.ischiadicus bij ratten, en ondersteund de theorie dat Org 2766 reinnervatie bevordert door stimulatie van sprouting bij regenerende axonen. De bovengenoemde resultaten werden niet gereflecteerd door enige verbetering in herstel van laryngeale functie, vergeleken met de placebo-behandelde katten. Org 2766 bleek geen significant effect op reinnervatie of functieherstel te hebben.

References

Anthony JP, Allen DB, Trabulsy PP, Mahdavian M, Mathes SJ. Canine laryngeal transplantation: Preliminary studies and a new heterotopic allotransplantation model. *Eur Arch Otorhinolaryngol* 1995;252:197-205.

Arnold GE. Physiology and pathology of the cricothyroid muscle. *Laryngoscope* 1961;71:687-753.

Arnold GE. Vocal rehabilitation of paralytic dysphonia. IX. Technique of intracordial injection. *Arch Otolaryngol* 1962;76:358-368.

Attali JP, Gioux M, Urtassun A, Henry C, Vital C, Traissac L. Vocal cord abduction rehabilitation by nervous selective anastomosis. *Laryngoscope* 1988;98:398-401.

Baldiserra F, Cantarella G, Marini G, Ottaviani F. Restoring abduction of paralyzed vocal cords in the cat using selective laryngeal reinnervation by phrenic motoneurons. *Laryngoscope* 1986;96:1399-1404.

Baldiserra F, Cantarella G, Marini G, Ottaviani F, Tredici G. Recovery of inspiratory abduction of the paralyzed vocal cords after bilateral reinnervation of the cricoarytenoid muscles by one single branch of the phrenic nerve. *Laryngoscope* 1989;99:1286-1292.

Ballance C. Results obtained in some experiments in which the facial and recurrent laryngeal nerves were anastomosed with other nerves: Some recovery of function. *Br Med J* 1924;2:349-354.

Barnes EB, Ballance C. Anastomosis of recurrent laryngeal to phrenic nerves. Some recovery of function. *Brit Med J* 1927;2:158-159.

Barnes C. Some experiments on nerve anastomoses. *Proc Mayo Clin* 1928;3:317-318.

Berke GS, Ye M, Block RM, Sloan S, Sercarz J. Orthotopic laryngeal transplantation: Is it time? *Laryngoscope* 1993;103:857-864.

Birchall M. Human laryngeal allograft: shift of emphasis in transplantation. *Lancet* 1998;351:539-340.

Bijlsma WA, Jennekens FGI, Schotman P, Gispen WH. Effects of corticotrophin (ACTH) on recovery of sensorimotor function in the rat: structure activity study. *Eur J Pharmacol* 1981;76:73-79.

Blalock A, Crowe SJ. The recurrent laryngeal nerves in dogs, experimental studies. *Arch Surg* 1926;12:95-116.

Boles R, Fritzell B. Injury and repair of the recurrent laryngeal nerves in dogs. *Laryngoscope* 1969;79:1405-1418.

Brondbo K, Hall C, Teig E, Dahl HA. Experimental laryngeal reinnervation by phrenic nerve implantation into the posterior cricoarytenoid muscle. *Acta Otolaryngol* 1987;103:339-344.

Broniatowski M, Kaneko S, Nose Y, Jacobs G, Tucker HM. Laryngeal pacemaker. II Electronic pacing of reinnervated posterior cricoarytenoid muscles in the canine. *Laryngoscope* 1985;95:1194-1198.

Capps FCW. Abductor paralysis in theory and practice since Semon. *J Laryngol Otol* 1958;72:1-31.

Clerf LH, Paralysis of the larynx. *Mich State Med Soc J* 1937;46:441-442,484.

Cohen MI. Phrenic and recurrent laryngeal discharge patterns and the Hering Breuer reflex. *Am J Physiol* 1975;228:1489-1496.

Colledge L. On the possibility of restoring movement of a paralyzed vocal cord by nerve anastomosis: An experimental enquiry. *Br Med J* 1925;1:547-548.

Colledge L, Ballance C. The first surgical treatment of paralysis of the diaphragm. *Br Med J* 1927;1:553-609.

Crumley RL. Mechanisms of synkinesis. *Laryngoscope* 1979;89:1847-1854.

Crumley RL, McCabe BF. Regeneration of the recurrent laryngeal nerve. *Otolaryngol Head Neck Surg* 1982a;90:442-447.

Crumley RL. Experiments in laryngeal reinnervation. *Laryngoscope* 1982b;92: No.9, part 2 (suppl no.30):1-27.

Crumley RL. Phrenic nerve graft for bilateral vocal cord paralysis. *Laryngoscope* 1983;93:425-428.

Crumley RL. Selective reinnervation of vocal cord adductors in unilateral vocal cord paralysis. *Ann Otol Rhinol Laryngol* 1984;93:351-356.

Crumley RL, Izdebski K. Voice quality following laryngeal reinnervation by ansa hypoglossi nerve transfer. *Laryngoscope* 1986;96:611-616.

Crumley RL. Laryngeal synkinesis: its significance to the laryngologist. *Ann Otol Rhinol Laryngol* 1989;98:87-92.

Crumley RL. Update: ansa cervicalis to recurrent laryngeal nerve anastomosis for unilateral laryngeal paralysis. *Laryngoscope* 1991;101:384-387.

Dedo HH. Electromyographic and visual evaluation of recurrent laryngeal nerve anastomosis in dogs. *Ann Otol Rhinol Laryngol* 1971;80:664-668.

Dekker A. Enhancement of peripheral nerve regeneration in the rat by a previous nerve injury and by treatment with neuropeptides. Thesis 1987a.

Dekker AJAM, Princen MM, De Nijs H, De Leede LGJ, Broekkamp CLE. Acceleration of recovery from sciatic nerve damage by the ACTH(4-9) analog Org 2766: different routes of administration. *Peptides* 1987b;8:1057-1059.

De Koning P, Neijt JP, Jennekens FGI, Gispen WH. Org 2766 protects from cisplatin induced neurotoxicity in rats, *Exp Neurol* 1987;97:746-750.

Dennis DP, Kashima H. Carbon dioxide laser posterior cordectomy for treatment of bilateral vocal cord paralysis. *Ann Otol Rhinol Laryngol* 1989;98:930-934.

Diamond AJ, Goldhaber N, Wu BL, Biller H, Sanders I. The intramuscular nerve supply of the posterior cricoarytenoid muscle of the dog. *Laryngoscope* 1992;102:272-276.

Doyle PJ, Brummett RE, Everts EC. Results of surgical section and repair of the recurrent laryngeal nerve. *Laryngoscope* 1967;77:1245-1254.

Doyle PJ, Everts E, Brummett R. Treatment of recurrent laryngeal nerve injury. *Arch Surg* 1968;96:517-520.

Doyle PJ, Westerberg BD, Chepeha DB, Schwarz DWF. Phrenic nerve reinnervation of the cat's larynx: a new technique with proven success. *Ann Otol Rhinol Laryngol* 1993;102:837-842.

Ducker T, Kauffman PD. Metabolic factors in surgery of peripheral nerves. In: Clinical Neurosurgery. Congress Neurological Surgeons. 1977:408-409.

Easton PA, Fleetham JA, DelaRocha A. Respiratory function after paralysis of the right hemidiaphragm. *Am Rev Resp Dis* 1983;127:125-128.

Edds MVJr. Experiments on partially deneurotized nerves. I. Absence of branching of residual fibres. *J Exp Zool* 1949;111:211-226.

Edds MVJr. Collateral nerve regeneration. *Quart Rev Biol* 1953;28:260-276.

Edwards PM, Kuiters RRF, Boer GJ, Gispen WH. Recovery from peripheral nerve transection is accelerated by local application of α-MSH by means of microporous accurel polypropylene tubes. *J Neurol Sci* 1986;74:171-176.

Elies W, Pulsakar A. Histological findings in arytenoid cartilages after long-lasting recurrent nerve paralysis. *J Laryngol Otol* 1983;97:949-957.

Ellenbogen BG, Gerber TG, Coon RL, Toohill RJ. Accessory muscle activity and respiration. *Otolaryngol Head Neck Surg* 1981;89:370-375.

Elsberg CA. Experiments on motor nerve regeneration and direct neurotization of paralyzed muscles by their own and foreign nerves. *Science* 1917;45:318-320.

Fackler CD, Penet GE, Bedell GN. Effect of unilateral phrenic nerve section on lung function. *J Appl Physiol* 1967;23:923-926.

Fata J, Malmgren L, Gacek R, Dum R, Woo P. Histochemical study of the posterior cricoarytenoid muscle reinnervation by a nerve-muscle pedicle in the cat. *Ann Otol Rhinol Laryngol* 1987;96:479-487.

Fex S. Functioning remobilization of vocal cords in cats with permanent recurrent laryngeal nerve paralysis. *Acta Otolaryngol* 1970;69:294-301.

Fink BR, Basek M, Epanchin V. The mechanism of opening of the human larynx. *Laryngoscope* 1956;66:410-425.

Fink BR. The human larynx: a functional study. Raven Press, New York, 1975.

Ford CN. Laryngeal injection techniques. In:Phonosurgery; Assessment and surgical management of voice disorders. CN Ford, DM Bless (Eds), Raven Press, New York, 1991:123-141.

Frazier CH. Anastomosis of the recurrent laryngeal nerve with the descendens noni. *JAMA* 1924;83:1637-1641.

Frazier CH, Mosser WB. Treatment of recurrent laryngeal nerve paralysis by nerve anastomosis. *Surg Gynaecol Obstet* 1926;43:134-139.

Fukuda H, Sasaki CT, Kirchner JA. Vagal afferent influences on the phasic activity of the posterior cricoarytenoid muscle. *Acta Otolaryngol* 1973;75:112-118.

Gacek RR, Lyon MJ. Fibre components of the recurrent laryngeal nerve in the cat. *Ann Otol Rhinol Laryngol* 1976;85:460-471.

Geterud A, Ejnell H, Stenborg R, Bake B. Long-term results with a simple surgical treatment of bilateral vocal cord paralysis. *Laryngoscope* 1990;100:1005-1008.

Gordon JH, McCabe BF. The effect of accurate neurorrhaphy on reinnervation and return of laryngeal function. *Laryngoscope* 1968;78:236-250.

Gutmann E, Young JZ. The reinnervation of muscle after various periods of atrophy. *J Anat* 1944;78:15-43.

Gwynn DG, Aitken JT. The formation of new motor endplates in mammalian skeletal muscle. *J Anat* 1966;100:111-126.

Hast MH. Studies on extrinsic laryngeal muscles. *Arch Otolaryngol* 1968:88:273-278.

Hengerer AS, Tucker HM. Restoration of abduction of the paralyzed canine larynx. *Arch Otol* 1973;97:247-250.

Hirose H, Ushijima T, Kobayshi T, Sawashima M. An experimental study of the contraction properties of the laryngeal muscles in the cat. *Ann Otol Rhinol Laryngol* 1969;78:297-306.

Hiroto I, Hirano M, Tomita H. Electromyographic investigations of human vocal cord paralysis. *Ann Otol Rhinol Laryngol* 1968;77:296-304.

Hoffman H. Local reinnervation in partially denervated muscle: a hist-physiological study. *Aust J Exp Biol Med Sci* 1950;28:383-397.

Hoover WB. Surgical procedures for the relief of symptoms of the paralysis of the recurrent laryngeal nerves. *Surg Clin N Am* 1953;33:879-885.

Horiuchi M, Sasaki CT. Cricothyroid muscle in respiration. *Ann Otol* 1978;78:386-391.

Horsely JA. Suture of the recurrent laryngeal nerve with report of a case. *Trans South Surg Gynaecol Assoc* 1909;22:161-170.

Isogai Y, Suzuki M, Saito S. Brainstem response evoked by the laryngeal reflex. In: Laryngeal function in phonation and respiration. Singular Publishing Group, San Diego. 1991; pp:71-79.

Isshiki N, Morita H, Okamura H, Hiramoto M. Thyroplasty as a new phonosurgical technique. *Acta Otolaryngology* 1974;78:451-457.

Isshiki N, Tanabe M, Sawada M. Arytenoid adduction for unilateral vocal cord paralysis. *Arch Otolaryngol* 1978;104:555-558.

Iwamura S. Functioning remobilization of the paralyzed vocal cord in dogs. *Arch Otolaryngol* 1974;100:122-129.

Jacobs IN, Sanders I, Wu BL, Biller HF. Reinnervation of the canine posterior cricoarytenoid muscle with sympathetic preganglionic neurons. *Ann Otol Laryngol* 1990;99:167-174.

Kelly WD. Phrenic nerve paralysis: special consideration of the accessory phrenic nerve. *J Thoracic Surg* 1950;19:923-928.

Kirchner JA. Laryngeal reflex systems. In: Laryngeal function in phonation and respiration. Singular Publishing Group, San Diego. 1991; pp:65-70.

Kluyskens P, Ringoir S. Follow up of a human larynx transplantation. *Laryngoscope* 1970;80:1244-1250.

Koning P de, Gispen WH. Org 2766 improves functional and electrophysiological aspects of regenerating sciatic nerve in the rat. *Peptides* 1987;8:415-422.

Kotby M, Haugen L. The mechanics of laryngeal function. *Acta Otolaryngol* 1970;70:203-211.

Lahey FH, Successful suture of recurrent laryngeal nerve for bilateral abductor paralysis with restoration of function. *Ann Surg* 1928;87:481-484.

Lahey FH, Hoover WB. Injuries to the recurrent laryngeal nerve and thyroid operations. *Ann Surg* 1938;108:545-562.

Langnickel R. Experimentelle Unterschungen zur Ankylose des Crico-Arytenoidgelenkes am Kaninchenkehlkopf. *Zeitschrift fur Laryngologie, Rhinologie, Otologie und ihre Grenzgebiete* 1973;52:67-65.

Lyons RM, Tucker HM. Delayed restoration of abduction in the paralyzed canine larynx. *Arch Otolaryngol* 1974;100:176-179.

Mahieu HF, van Lith-Bijl JT, Groenhout C, Tonnaer JADM, de Wilde P. Selective laryngeal abductor reinnervation in cats using a phrenic nerve transfer and Org 2766. *Arch Otolaryngol Head Neck Surg* 1993;119:772-776.

Mahieu HF, Norbart T, Snel F. Laryngeal framework surgery for voice improvement. *Rev Laryngol Otol Rhinol* 1996:117:189-197.

Marie JP, Dehisdin D, Ducastelle T, Senant J. Selective Reinnervation of the abductor and adductor muscles of the canine larynx after recurrent nerve paralysis. *Ann Otol Rhinol Laryngol* 1989;98:530-536.

Mathew OP, Sant'Ambrogio FB, Woodson GE, Sant'Ambrogio G. Respiratory activity of the cricothyroid muscle. *Ann Otol Rhinol Laryngol* 1988;97:680-687.

McCall JW, Hoerr NL. Reinnervation of a paralyzed vocal cord. *Laryngoscope* 1946:56:527-535.

McNeil BJ, Wechselbaum R, Pauker SG. Speech and survival. Tradeoffs between quality and quantity of life in laryngeal cancer. *N Eng J Med* 1981;305:982-987.

Miehlke A. Rehabilitation of vocal cord paralysis. *Arch Otolaryngol* 1974;100:431-441.

Mikaelian D, Lowry L, Sataloff R. Lipoinjection of unilateral vocal cord paralysis. *Laryngoscope* 1991;101:465-468.

Morledge, DR, Lauvstad WA, Calcaterra TC. Delayed reinnervation of the paralyzed larynx. An experimental study in the dog. *Arch Otolaryngol* 1973;97:291-293.

Mu L,Yang S. Electromyographic study on end-to-end anastomosis of the recurrent laryngeal nerve in dogs. *Laryngoscope* 1990;100:1009-1017.

Murakumi Y, Kirchner JA. Vocal cord abduction by regenerated recurrent laryngeal nerve. *Arch Otolaryngol* 1971;94:64-68

Murakumi Y, Kirchner JA. Respiratory movement of the vocal cords, an electromyographic study in the cat. *Laryngoscope* 1972;82:454-467.

Nahm I, Shin T, Watanabe H, Maeyama T. Misdirected regeneration of injured recurrent laryngeal nerve in the cat. *Am J Otolaryngol* 1993;14:43-48.

Neal D, Cummings CW, Sutton D. Delayed reinnervation of unilateral vocal cord paralysis in dogs. *Otolaryngol Head Neck Surg* 1981;89:608-612.

Negus VE. Certain anatomical and physiological considerations in paralysis of the larynx. *Proc R Soc Med* 1947;40:849-53.

Negus VE. The comparative anatomy and physiology of the larynx. Heineman, London, 1949.

Ogura JH, Kawasaki M, Takenouchi S, Yagi M. Replantation and transplantation of the canine larynx. *Ann Otol Rhinol Laryngol* 1966;75:295-313.

Ossoff RH. Endoscopic laser arytenoidectomy for vocal cord paralysis. *Laryngoscope* 1984;94:1293-1297.

Rice DH. Laryngeal reinnervation. *Laryngoscope* 1982;92:1049-1059.

Rice DH, Owens O, Burstein F, Verity A. The nerve muscle pedicle, A visual, electromyographic and histochemical study. *Arch Otolaryngol* 1983a:109:233-234.

Rice DH, Burstein FD. Restoration of physiologic vocal fold abduction with the ansa cervicalis nerve. *Arch Otolaryngol* 1983b;109:480-481.

Robotham J. A physiological approach to hemidiaphragm paralysis. *Crit Care Med* 1979;7:563-565.

Saito A, Zacks SI. Fine structure observations of denervation and reinnervation of neuromuscular junctions in mouse foot muscle. *J Bone Joint Surgery* 1969;51:1163-1178.

Sasaki CT, Fukuda H, Kirchner JA. Laryngeal abductor activity in response to varying ventilatory resistance. *Trans Am Acad Ophthalmol Otolaryngol* 1973;77:403-410.

Sasaki CT, Suzuki M. Laryngeal reflexes in cat, dog and man. *Arch Otolaryngol* 1976;102:400-402.

Shimazaki S. Experimental study on the neurosurgical treatment of recurrent laryngeal nerve paralysis. *Pract Otol Kyoto* 1957;50:562-581.

Silver CE, Rosen RG, Dardik I, Eisen H, Schwibner BH, Som ML. Transplantation of the canine larynx. *Ann Surg* 1970;172:142-150.

Silver CE, Rosen RG. Function of transplanted and denervated larynges. II Arytenoidectomy with sensory reinnervation. *Arch Otolaryngol* 1974;99:100-101.

Siribodhi C, Sunderland W, Atkins JP, Bonner FJ. Electromyographic studies on laryngeal paralysis and regeneration of laryngeal motor nerves in dogs. *Laryngoscope* 1963;73:148-163.

Spiegel J, Sataloff R. The treatment of vocal fold paralysis with injectable collagen: clinical concerns. *J Voice* 1987:1;119-121.

Spiro RG. Nature of glycoprotein components of basement membranes. *Ann NY Acad Sci* 1978;312:106-119.

Starzl TE, Demetris AJ, Vanthiel D. Liver transplantation (Second of Two Parts). *N Eng J Med* 1989;321:1092-1099.

Strand FL, Kung TT. ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. *Peptides* 1980;1:135-138.

Strome M, Strome S. Laryngeal transplantation, the future. *Otolaryngol Clin N Am* 1991;24:1358-1359.

Strome S, Sloman-Moll E, Samonte BR, Wu J, Strome M. Rat model for a vascularized laryngeal allograft. *Ann Otol Rhinol Laryngol* 1992;101:950-953.

Sunderland S, Swaney WE. The interneural topography of the recurrent laryngeal nerve in man. *Anat Rec* 1952;114:411-426.

Sunderland S. Nerve and nerve injuries. Churchill Livingstone, Edinburgh, Scotland, and London, England 1972 pp.127-135.

Suzuki M, Kirchner JA. Sensory fibers in the recurrent laryngeal nerve. *Ann Otol Rhinol Laryngol* 1969a;78:21-31.

Suzuki M, Kirchner JA. The posterior cricoarytenoid as an inspiratory muscle. *Ann Otol Rhinol Laryngol* 1969b;78:849-863.

Suzuki M, Kirchner JA, Murakumi Y. The cricothyroid as a respiratory muscle. Its characteristics in bilateral laryngeal nerve paralysis. *Ann Otol Rhinol Laryngol* 1970;79:976-983.

Suzuki M, Sasaki CT. Effect of various sensory stimuli on reflex laryngeal adduction. *Ann Otol Rhinol Laryngol* 1977;86:30-36.

Taggart JP. Laryngeal reinnervation by phrenic nerve implantation in dogs. *Laryngoscope* 1971;81:1330-1336.

Tashiro T. Experimental studies on the reinnervation of the recurrent laryngeal nerve in dogs. *Laryngoscope* 1972;82:225-236.

Thumfart WF. Electrodiagnosis of laryngeal nerve disorders. *Ear Nose Throat J* 1988;67:380-393.

Tonnaer JADM, Schuijers GJPT, Van Diepen HA, Peeters BWMM. Enhancement of regeneration by Org 2766 after nerve crush depends on the type of neural injury. *Eur J Pharmacol* 1992;214:33-37.

Tucker HM, Harvey J, Ogura JH. Vocal cord remobilization in the canine larynx. *Arch Otolaryngol* 1970;92:530-533.

Tucker HM, Ogura JH. Vocal cord remobilization in the canine larynx: an histologic evaluation. *Laryngoscope* 1971:81:1602-1606.

Tucker H. Laryngeal transplantation: current status 1974. *Laryngoscope* 1974;84:787-796.

Tucker HM. Human laryngeal reinnervation. *Laryngoscope* 1975;85:769-779.

Tucker HM. Human Laryngeal reinnervation : Long-term experience with nerve-muscle pedicle technique. *Laryngoscope* 1978;88:598-604.

Tucker HM. Laryngeal reinnervation for unilateral vocal cord paralysis. *Ann Otol Rhinol Laryngol* 1981;90:457-459.

Tucker HM. Combined laryngeal framework medialization and reinnervation for unilateral vocal fold paralysis. *Ann Otol Rhinol Laryngol* 1990;99:778-780.

Tucker HM. Simultaneous medialization and reinnervation for unilateral vocal fold paralysis. *Operative Techniques in Otolaryngology-Head and Neck Surgery* 1993;4(3):183-185.

van Lith-Bijl JT, Mahieu HF, Stolk RJ, Tonnaer JADM, Groenhout C, Konings PNM. Laryngeal abductor function after laryngeal nerve injury in cats. *Arch Otolaryngol Head Neck Surg* 1996a;122:393-396.

van Lith-Bijl JT, Mahieu HF. Is laryngeal transplantation feasible? In: Surgery and prosthetic voice restoration after total and subtotal laryngectomy. Algaba J (Ed). Elsevier Science.1996b; pp 35-39.

van Lith-Bijl JT, Mahieu HF, Stolk RJ, Tonnaer JADM, Groenhout C, Konings PNM. Selective laryngeal reinnervation with separate phrenic and ansa cervicalis nerve transfers. *Arch Otolaryngol Head Neck Surg* 1997a;123:406-411.

van Lith-Bijl JT, Mahieu HF. Laryngeal reinnervation surgery - results of a selective approach in an animal study. *Indian J Otolaryngol Head Neck Surg* 1997b;49:203-208.

van Lith-Bijl JT, Mahieu HF. Selective laryngeal reinnervation surgery: an animal study. In: Advances in laryngology in Europe. Kleinsasser O, Glanz H, Olofsson J (Eds). Elsevier Science. 1997c; pp 440-446.

van Lith-Bijl JT, Stolk RJ, Tonnaer JADM, Groenhout C, Konings PNM, Mahieu HF. Laryngeal abductor reinnervation with a phrenic nerve transfer after a 9-month delay. *Archives of Otolaryngol Head & Neck Surg* 1998;124:393-398.

Van der Zee CEEM, Gerritsen van der Hoop R, Gispen WH. Beneficial effect of the peptide Org 2766 ($ACTH_{(4-9)}$ analog) in the treatment of neuropathy in streptozotocin-diabetic rats. *Diabetes* 1989;38:225-230.

Verhaagen J, Edwards PM, Jennekens FGI, Schotman P, Gispen WH. Early effect of an ACTH4-9 analog (ORG 2766) on regenerative sprouting demonstrated by the use of neurofilament-bindingantibodies isolated from a serum raised by α-MSH immunization. *Brain Res* 1987;404,142-150.

Vilkman EA, Pitkänen R, Suominen H. Observations on the structure and the biomechanics of the cricothyroid articulation. *Acta Otolaryngol (Stockh)* 1987;103:117-126.

Weed DT, Chongkolwatana C, Kawamura Y, Burkey BB, Netterville JL, Ossoff RH, Zealear DL. Reinnervation of the allograft larynx in the rat laryngeal transplant model. *Otolaryngol Head Neck Surg* 1995;113:517-29.

Wu BL, Sanders I, Mu L, Biller H. The Human communicating nerve. *Arch Otolaryngol Head Neck Surg*. 1994;120:1321-1328.

Wyke BD. Effects of anaesthesia upon intrinsic laryngeal reflexes. *J Laryngol* 1968;82:603-611.

Zealear DL. Rainey CL, Tanabe T, Jerles ML, Herzon GD. Technical approach reanimation of the chronically denervated larynx by means of functional electrical stimulation. *Ann Otol Rhinol Laryngol* 1994;103:705-712.

Zheng H, Li Z, Zhou S, Cuan Y, Wen W, Lan J. Experimental study on reinnervation of vocal cord adductors with the ansa cervicalis. *Laryngoscope* 1996;106:1516-1521.

Zheng H, Li Z, Zhou S, Cuan Y, Wen W. Update: Laryngeal reinnervation for unilateral vocal fold paralysis with the ansa cervicalis. *Laryngoscope* 1996;106:1522-1527.

Dankwoord

Allereerst wil ik mijn speciale dank uiten aan prof.dr. H.F. Mahieu. Beste Hans, inmiddels kennen wij een samenwerkingsperiode van ruim acht jaar waarbij dit onderzoeksproject een sleutelrol heeft gespeeld en ik veel van jou heb mogen leren. Van beide kanten is er veel geduld nodig geweest om tot dit proefschrift te komen. Ik heb er steeds bewondering voor gehad dat je ondanks je zeer drukke werkzaamheden steeds voldoende belangstelling en concentratie wist op te brengen om mijn onderzoekswerk serieus te begeleiden. Dat je niet snel tevreden bent is iets wat op den duur wel wordt beloond en waar ik dan ook nu van profiteer. Bedankt voor je inzet dit project tot een goed einde te brengen.

Mijn dank aan prof.dr. G.B. Snow, mijn opleider, voor zijn kritische beoordeling van de manuscripten vòòr verzending voor publicatie.

Voor het uitoefenen van de dierenexperimenten en verzorging van de dieren is de hulp van Joop Grimbergen essentieel geweest. Joop, dank voor je inzet en betrokkenheid. Robin Stolk wil ik bedanken voor zijn medewerking gedurende de voorbereidingen en het uitvoeren van de experimenten.

Fred Snel, Fred, je technische hulp en doeltreffendheid, bij de opzet van het onderzoek, en je voortdurende bereidheid te helpen bij problemen heb ik zeer gewaardeerd.

De firma Organon nv. heeft de uitvoering van het onderzoeksproject financieel ondersteund. Mijn dank voor de medewerking en belangstelling van Jeroen Tonnaer, Cees Groenhout en Pierre Konings. De histologische analyses zijn verricht door Geert Schuijers.

De statistische bewerking is uitgevoerd door Hilde Tobi.

De illustraties in het proefschrift zijn gemaakt door Ruud de Haan.

Al mijn directe collegae wil ik bedanken voor hun steun waardoor het mogelijk is geweest bij de opleiding KNO, toch steeds deze onderzoekswerkzaamheden voort te zetten.

Last but not least, wil ik Jan, mijn echtgenoot, bedanken voor zijn jarenlange geduld, mij te delen met dit langdurige onderzoek en dit te blijven doen toen we Floris en Pleuntje erbij kregen.

Lieve Floris en Pleuntje, later zal mamma het promoveren nog eens aan jullie uitleggen.

Curriculum Vitae

The author was born on 17th October 1964 in Blantyre, Malawi. She went to St. Andrews Primary and Secondary School in Blantyre. Having passed the G.C.E. A-levels in Mathematics, Biology, Physics an Chemistry in 1982, she left for the Netherlands to study medicine at the Rijks Universiteit Groningen.
In 1989 she graduated as a doctor (artsexamen). In the meantime some time was spent traveling the world being an air-hostess with KLM, Royal Dutch Airlines.
Having graduated she soon started research work in the E.N.T. Department in the Groningen University Hospital, under supervision of Hans Mahieu, involving clinical evaluation of the low resistant Groningen button tracheo-esophageal valve prosthesis, for post-laryngectomy voice rehabilitation. Meanwhile a laryngeal reinnervation pilot study was initiated in cats.
From 1991 the author continued the laryngeal reinnervation research project in the clinical animal laboratory of the Vrije Universiteit Amsterdam. From 1991 to 1996 she specialized in Otorhinolaryngology. She is now working as an E.N.T. specialist in Flevo Hospital in Almere.

She is married to Jan van Lith and they have two children, Floris and Pleuntje and their younger brother or sister is expected at the end of October.