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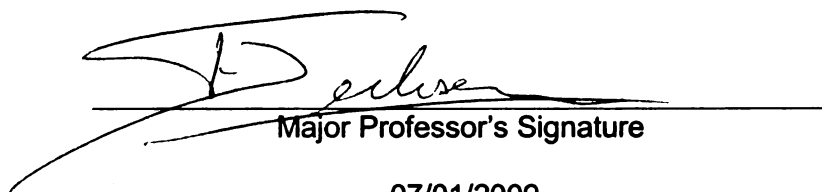
**IMPROVEMENTS TO THE NERVE MUSCLE PEDICLE
GRAFT TECHNIQUE FOR THE TREATMENT OF LEFT
RECURRENT LARYNGEAL NEUROPATHY IN THE HORSE**

presented by

Philip Andrew Cramp

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Large Animal Clinical Sciences



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THE TREATMENT OF LEFT RECURRENT LARYNGEAL NEUROPATHY IN THE
HORSE

By

Philip Andrew Cramp

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Large Animal Clinical Sciences

2009

ABSTRACT

IMPROVEMENTS TO THE NERVE MUSCLE PEDICLE GRAFT TECHNIQUE FOR THE TREATMENT OF LEFT RECURRENT LARYNGEAL NEUROPATHY IN THE HORSE

By

Philip Andrew Cramp

Five larynges were harvested from horses free from upper airway disease. Using increments of 0.98 N, a dead-weight force generator applied a force of 0 N through 14.70 N for 1 minute each to the left muscular process at 0, 10, 20, 30, 40, 50, 60, and 70 degree angles. The rima glottis was digitally photographed one minute after each force had been applied. Increasing force from 0 N to 14.70 N progressively and significantly increased the length of all lines and right to left angle quotient, indicating abduction. Applying forces in the direction of 0 to 30 degrees, which correspond with the direction of pull exerted by the lateral compartment of the cricoid arytenoideus dorsalis muscle, resulted in a significantly greater degree of laryngeal abduction.

Four horses with induced left laryngeal hemiplegia were treated with nerve muscle pedicle graft. Two horses were controls and 2 were subjected to electrostimulation of the first cervical nerve. Inspiratory pressure measurements, upper airway videoendoscopy on the treadmill and histology at post mortem were used to assess degree of improvement and effect with or without electrostimulation. No conclusions could be made due to technical complications.

DEDICATION

To my parents Dudley and Andrea Cramp, who have been a source of tremendous support and have made it possible for me to realize my ambition of becoming a veterinary surgeon.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr Fred Derksen for his advice and support. Throughout this project he has kept me on the 'straight and narrow' introducing me to, and educating me with the principles of scientific research. In particular I would like to thank him for his enthusiasm even when things were not going well, his positive nature is a huge resource. I would like to acknowledge Dr John Stick and Dr Ian Fulton for their surgical expertise, advice and assistance.

This project would not have been possible without the Pulmonary Laboratory at Michigan State University and I would like to highlight the influence and expertise of Dr Robinson, who has provided a great deal of guidance throughout this project. Furthermore, I would like to thank Cathy Berney, Sue Eberhart-Wisner and Heather de-Feijter Rupp for all of their technical assistance and support, without them this would not have been possible. I would also like to thank Ashley and Jenny for their good sense of humor at five o'clock in the morning helping me to electrostimulate the horses in the depths of a Michigan winter. Drs Stephanie Valberg and Kurt Williams also deserve thanks for their expertise in the histologic assessment of the nerves and muscles. I would like to acknowledge the financial support of the Freeman Fund and the Matilda R Wilson Endowment Fund for funding this research.

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KEY TO ABBREVIATIONS

RLN	Recurrent Laryngeal Neuropathy
LH	Laryngeal Hemiplegia
CAD	Cricoid Arytenoideus Dorsalis Muscle
NMP	Nerve Muscle Pedicle
ILH	Idiopathic Laryngeal Hemiplegia
EMG	Electrical Myography
CAL	Cricoid Arytenoideus Lateralis Muscle
MRI	Magnetic Resonance Imaging
CT	Computed Tomography
Nd:YAG	Neodymium Yttrium Aluminium Garnet Laser
PDS	Polydioxanone Suture Material
N	Newtons
RLQ	Right-to-left Quotient
NMC	Neuromuscular Compartment
i.v.	Intravenous
i.m.	Intramuscular
b.i.d.	Twice daily

s.i.d. Once daily

NADH Nicotinic Acid

Adenine Dinucleotide Dehydrogenase

1. Introduction

Recurrent laryngeal neuropathy (RLN) has a number of synonyms. It is also referred to as idiopathic laryngeal hemiplegia, laryngeal paralysis and more colloquially as “roaring”. For the purposes of this thesis, I shall be referring to this disease as RLN.

Recurrent laryngeal neuropathy is an important problem in the equine industry and is one of the most common causes of poor performance (Dixon *et al.* 2001; Russell and Slone 1994). The incidence of LH, as high as 35% in draft breeds (Brakenhoff *et al.* 2006), has been reported to range from 2.6 to 11% in lighter breeds (Lane 1987; Morris and Seeherman 1990; Raphel 1982).

Whilst the exact etiopathogenesis of the disease remains elusive, the term RLN refers to a unilateral (typically the left side) paralysis of the cricoid arytenoideus dorsalis muscle (CAD) resulting from a neuropathy of the left recurrent laryngeal nerve (Duncan *et al.* 1978; Duncan *et al.* 1974). During exercise, under the influence of increased negative inspiratory pressure, the paralyzed left arytenoid cartilage cannot be abducted and collapses into the airway, causing obstruction and noise production (Derksen *et al.* 2001; Morris and Seeherman 1990; Shappell *et al.* 1988).

Regardless of the cause, treatment is required to improve athletic performance. A multitude of treatments have been used including tracheostomy (Cook 1970) ventriculectomy (Hobday 1935), ventriculocorpectomy (Shappell *et al.* 1988), arytenoidectomy (Haynes *et al.* 1984), laryngoplasty (Marks *et al.* 1970) and neuromuscular pedicle graft (Ducharme *et al.* 1989; Fulton *et al.* 2003) alone or in combination. Currently, laryngoplasty with or without a ventriculocorpectomy is the treatment of choice despite a high complication and failure rate. The reported complications associated with laryngoplasty include nasal discharge of food, chronic coughing, and prosthesis failure (Hawkins *et al.* 1997; Russell and Slone 1994). Although efforts have been made to modify the technique, it still suffers from a number of these potentially devastating complications (Rossignol *et al.* 2006; Schumacher *et al.* 2000).

Laryngeal reinnervation is used to treat people with laryngeal denervation (Kingham *et al.* 2006; Su *et al.* 2007; Tucker 1989; Tucker and Rusnov 1981). Fulton *et al.* (1991) described the use of the first cervical nerve that supplies the omohyoideus muscle in a neuromuscular pedicle (NMP) graft technique in horses with laryngeal hemiplegia (Fulton *et al.* 1991; Fulton *et al.* 2003). The technique demonstrates the same level of success as the laryngoplasty but suffers from fewer complications and is a more physiologic treatment.

Three to 4 pedicles are usually available for transplantation in an individual horse, but the optimal location for placement of the pedicles within the CAD muscle is currently

unknown. Recently, an elegant study described the neuroanatomy of the equine CAD muscle (Cheetham *et al.* 2008). There is a medial and lateral neuromuscular compartment and the lateral compartment plays a greater role in abduction of the arytenoid cartilage. Therefore, optimal placement of neuromuscular pedicle grafts may improve surgical efficacy, including improved laryngeal abduction and shortening of the recovery time.

Regardless, the procedure has failed to gain widespread acceptance due to the long recovery time associated with the process of reinnervation; currently it is believed that about 9 months are required before a horse will be returned to normal respiratory soundness for athletic endeavor (Fulton *et al.* 2003). It is important to try and develop techniques to shorten this time period.

Electrostimulation of skeletal muscle after denervation has been studied extensively in humans and laboratory animals (Gondin *et al.* 2006; Maffiuletti *et al.* 2006; Marqueste *et al.* 2004; Poortmans and Wyndaele 2002; Vitenzon *et al.* 2005). Within 5 weeks, denervated muscle has a dramatic loss of size and ability to contract and carry out its function. For example, within 5 weeks, denervated extensor digitorum longus muscles of rats lose 66% of mass, 91% of force, and 76% of fiber cross-sectional area (Dow *et al.* 2004). Electrostimulation of these muscles can completely prevent these impairments (Dow *et al.* 2004). Atrophy of denervated muscle impairs its ability to become reinnervated (Cole and Gardiner 1984; Fu and Gordon 1995); therefore the process of electrostimulation may aid the reinnervation process. This possibility has

important implications in equine LH, where the denervated CAD muscle is responsible for the loss of upper airway function.

The electrostimulation protocol, muscle fiber type, and species differences have important effects on muscle responses to stimulation (Dow *et al.* 2004). Generally, frequent submaximal stimulation encourages slow fiber type growth, while less frequent maximal contractions encourage fast twitch fibers, muscle fiber hypertrophy, and faster force recovery. For example, in the denervated extensor digitorum longus muscle of rats, a typical fast twitch muscle, near normal force was maintained with 100 contractions per day for 5 weeks, while 800 contractions per day caused muscle damage (Dow *et al.* 2004). In another study involving denervated (for 6 to 24 weeks) human quadriceps muscle, a mixed fiber type muscle, 80 contractions per week for 8 weeks were sufficient to generate a 20% increase in muscle mass (Dudley *et al.* 1999). The equine CAD muscle is a mixed fast (55%) and slow twitch (45%) skeletal muscle (Hoh 2005), similar to the human quadriceps muscle (Green *et al.* 1999).

To assist the surgeon in choosing the optimal placement of nerve-muscle pedicle grafts, one of the aims of my thesis research was to systematically determine force-laryngeal abduction relationships at various locations within the CAD muscle. This study provided information for a pilot study, which was designed to investigate if, in horses with LH, postoperative electrostimulation of the reinnervated muscle allows earlier return to athletic performance without risk of coughing, aspiration, and respiratory infection.

In the remainder of this Thesis I shall review equine laryngeal anatomy and function, RLN, its treatment and report on the findings of my research.

2. Anatomy of the Equine Larynx

The larynx is a complex anatomical structure that connects the pharynx to the tracheobronchial tree. It is a short tube that is responsible for not only the transit of air from the atmosphere and nasopharynx to the trachea and lungs, but it regulates the volume of air inspired and expired, prevents aspiration during swallowing and also is the organ responsible for phonation. It is composed of cartilage structures, ligament, muscles and tendons as well as afferent and efferent nerves, which determine how this complicated little organ will carry out its diverse responsibilities.

The larynx is situated ventrally and relatively superficially (Getty, 1975). It is positioned dorsal to the sternohyoideus and omohyoideus muscles, and ventral to the pharynx and proximal extent of the esophagus. In a normal head carriage, the rostral half of the larynx lies between the mandibular rami and it is flanked on either side by the stylohyoideus muscle and the digastricus muscle, the parotid salivary glands and the linguofacial vein (Getty, 1975). The larynx is connected to the tongue and hyoid apparatus via the basihyoid and thyrohyoid bones.

There are three unpaired cartilages that make up the framework of the larynx, namely; the cricoid, the thyroid and the epiglottic along with three paired sets of cartilages, namely; the arytenoid, the corniculate and the cuneiform cartilages, which are fused

with the epiglottic cartilage (Getty, 1975). The epiglottic cartilage is the most rostral and is described as an oblancoolate leaf (Getty, 1975). The base is referred to as a small stalk that is embedded between the root of the tongue, the basihyoid, and the body of the thyroid cartilage. Attached to either side of the base of the epiglottic cartilage are the paired cuneiform cartilages. These are thin elastic bars that originate at the base of the epiglottic cartilage and support the mucosal folds passing from the epiglottis to the arytenoids (Getty, 1975). Moving caudally, the thyroid cartilage is the largest of the laryngeal cartilages (Getty, 1975). It consists of two lateral plates that fuse medially to a varying degree producing a slight prominence. It is related dorsally to the base of the epiglottic cartilage via an elastic ligamentous attachment. The rostral cornu of the thyroid cartilage articulates with the thyrohyoid bone. The caudal cornu of the thyroid cartilage articulates with the cricoid cartilage, the ventral border joins the body rostrally, and diverges caudally to form a triangle, which is covered by the cricothyroid membrane (Getty, 1975). The signet ring shaped cricoid cartilage is the next structure encountered as we move caudally. The dorsal aspect of the cricoid cartilage possesses a median ridge and it is within the shallow concaves of the dorsal surface that the cricoid arytenoideus dorsalis muscle is situated. The caudal aspect of the dorsal cricoid cartilage is thin and overhangs the first tracheal ring; the rostral aspect is much thicker and possesses two facets for the arytenoid cartilage articulations. Ventrally, there is attachment of the cricothyroid membrane and the cricoarytenoideus lateralis (Getty, 1975). Caudally from the ventral aspect, the cricoid cartilage is attached to the first ring of the trachea by the cricotracheal membrane. The paired arytenoid cartilages on either side of the rostral aspect of the cricoid cartilage,

just medial to the thyroid cartilage and are best described as pyramidal in form (Getty, 1975). Each arytenoid cartilage possesses three processes; a vocal process, which attaches to the vocal cord; a muscular process, which extends laterally and is the site of insertion for the cricoid arytenoideus dorsalis; and a corniculate process, which is orientated caudomedially and forms the dorsomedial and dorsolateral boundaries of the rima glottidis. The corniculate processes are also named the corniculate cartilages.

These cartilages are joined together by synovial diarthrodial joints (cricothyroid articulations, cricoarytenoid articulations and thyrohyoid articulations) all of which possess a thin synovial capsule. The cricotracheal ligament is elastic and attaches the first ring of the trachea to the cricoid cartilage, the cricothyroid ligament is elastic and is found between the rostral border of the ventral aspect of the cricoid cartilage and the caudal border of the lamina of the thyroid cartilage. The ventral part of this ligament is the cricothyroid membrane. The cricoarytenoid ligament supports the ventral aspect of the capsule and the transverse arytenoid ligament connects the dorsomedial angles of the opposing arytenoid cartilages. The thyroepiglottic and hyoepiglottic ligaments run from the base of the epiglottic cartilage to the inner surfaces of the thyroid cartilages and to the basihyoid and lingual process of the hyoid bone respectively. The vestibular and vocal ligaments run from the arytenoid cartilages ventrad.

The muscles of the larynx can be divided into two groups, namely intrinsic and extrinsic (Getty, 1975). The extrinsic muscles of the larynx are made up of the thyrohyoideus and hyoepiglotticus muscles, which receive their efferent nervous

supply from the hypoglossal nerve and the sternothyrohyoideus and omohyoideus that receive their innervation via branches of the first and second cervical nerves. These muscles are responsible for location and movement of the larynx in its entirety. The intrinsic muscles consist of the cricothyroideus, cricoarytenoideus dorsalis, cricoarytenoideus lateralis, arytenoideus transversus and the thyroarytenoideus. These muscles are responsible to the movement of the cartilages of the larynx, below is a description of their movement (taken from Getty (1975) in Sisson's and Grossman's The Anatomy of Domestic Animals, Volume 1, page 509):

“The cricothyroideus tenses the vocal fold and ligament by approximating the ventral parts of the cricoid and the thyroid cartilages, thereby increasing the diameter of the rima glottidis. This action also has the effect of adducting the vocal folds. The thyroarytenoideus, arytenoideus transverses, and the cricoarytenoideus lateralis adduct the vocal processes of the arytenoid cartilages and narrow the rima glottidis. The cricoarytenoideus dorsalis and the arytenoideus transverses abduct the vocal processes.”

The cricothyroideus muscle is supplied by motor fibers in the external branch of the cranial laryngeal nerve whereas all of the other intrinsic musculature is supplied by motor fibers in the recurrent laryngeal nerve (Getty, 1975).

The mucus membrane of the larynx is contiguous with the mucus membrane of the laryngopharynx and the trachea (Getty, 1975). The aryepiglottic folds are formed by

the membranous reflection from the borders of the epiglottic cartilage. Within the larynx, on either side of the laryngeal wall, the mucus membrane covers the vocal ligament and the vocalis muscle forming the basis of the vocal cord. Furthermore, this mucus membrane forms another fold as it covers the cuneiform cartilage, the vestibular ligament and the underlying part of the vestibularis muscle, this is commonly known as the vestibular fold. Lying between this fold and the vocal cord is a 2.5-5 cm deep reflection known as the lateral ventricle, or laryngeal saccule.

The blood supply to the larynx arrives in the form of the caudal laryngeal arteries and branches from the ascending pharyngeal arteries, which can arise from either the cranial thyroid artery or the common carotid artery (Getty, 1975). The venous drainage is into the external jugular vein. The lymphatic vessels draining the larynx run into the retropharyngeal and cranial deep cervical lymph nodes (Getty, 1975).

3. Equine Recurrent Laryngeal Neuropathy

a. A Historical Disease

One of the first complete reports on RLN was published in 1882 by Fleming, it was entitled Laryngismus paralytica (“roaring”) and since then there has been much in the literature about this common equine disease. However, in a review article by MacQueen in 1896 reference is made to earlier reports of “roaring” dating back to 1664 and 1807 (Dupuy). Indeed, in the later reference, an association between degeneration of the muscles on the left side of the larynx and horses that “roared” had been established. So, even at that time it was recognized by veterinarians that this disease arose from a paralyzed larynx that affected function and produced a “roaring” noise. Furthermore, it was appreciated that this “roaring” noise was a symptom of the disease and not in itself a disease. In Flemming’s article in 1882 he makes mention of the observation made in 1866 by Gunther that 96% of horses with clinical signs of chronic “roaring” had wasting of the muscles of the left side of the larynx.

In 1905, Professor Wortley provided a review of “Roaring and Whistling”. He noted that this was a symptom and that chronic roaring related to wasting of the dilator muscles of the larynx. It was suggested that this would be the result of

strangles, influenza or some affection of the chest that results in damage and paralysis of the nerve supply the affected muscles. He goes on to commentate on the fact that it is invariably the left side (left recurrent branch of the pneumogastric nerve) that is affected because it winds around the base of the aorta. Whilst Professor Wortley could not discover any microscopic differences between nerves he does make the observation that the right side is far less frequently affected. Furthermore, he also notes that the disease is seldom seen in equidae less than 14 hands and that height and length of the neck are proportional to the risk of acquiring the disease. This is a statement that echoed the sentiments Flemming and McCall, who in 1889 noted that the large breeds such as saddle horses and draught horses are most commonly affected and that it usually occurs in younger horses (three to five years of age).

Despite the fact that Flemming (McCall, 1889) stated that during inspiration, the left lateral ventricle fills with air and billows into the lumen of the larynx and that during exercise the arytenoid cartilage may also contribute in affected horses diagnosis was based on clinical observation. If noise was heard at exercise by an experienced ear it was considered to be diagnostic. Symptoms such as grunting were a common finding among clinically affected roarsers and something that horse dealers became very familiar with. Often times, threatening an animal with a stick to see if they grunt in fear, and pinching the throat to evoke a cough were used to help diagnose the condition. Professor Wortley (1905) comments on the characteristic nature of the cough. He states that the clinical “roarer” will have a

deep cough, which is a mixture of a groan and a cough, the subtlety of which can be easily appreciated by an experienced listener.

Due to the fact that a number of celebrated racehorses were susceptible to this disease, treatment was sought. Between the years of 1860 and 1906, a number of surgical treatments were attempted. These included vocal cordectomy, ventriculectomy and partial and total arytenoidectomy. Initially, these attempts were unsuccessful due to infection and aspiration pneumonia and it was not until 1905 when Professor Wortley suggested that whilst arytenoidectomy cannot be considered as a success it should also not be regarded as an unqualified failure. However, at this time tracheostomy was still considered to be the treatment of choice despite its recognized limitations of exuberant granulation tissue formation and irritation. In addition to surgery, management in 1905 consisted of keeping horses in high condition, the application of “counter-irritants” to the throat, such as mustard or turpentine liniment, not to mention, of course, the early application of nux vomica!

In 1906, Williams began treating horses with ventriculectomy and after five years of modifications reported that 71% of roasters produced no noise after the procedure. Sir Frederick Hobday reported on 405 cases in 1911 and in 1935 he commented that 85-95% of roasters were returned to useful function after a ventriculectomy, however only 20% produced no noise. So successful was the procedure perceived to be that its popularity in the United Kingdom led the

ventriculectomy being known, as it is still today, as the “Hobday” procedure. However, a treatment was born and from this point modifications continued and improvements made culminating in the Laryngoplasty by Marks (1970).

b. The Present Day

i. Incidence

This has been covered in part in the Introduction section of this Thesis, however, to reiterate; RLN is an important problem in the equine industry and is one of the most common causes of poor performance (Dixon *et al.* 2001; Russell and Slone 1994). The incidence of LH, as high as 35% in draft breeds (Brakenhoff *et al.* 2006), has been reported to range from 2.6 to 11% in lighter breeds (Lane 1987; Morris and Seeherman 1990; Raphael 1982). However, in the study by Brakenhoff *et al.* (2006) only 17% of Clydesdales were affected compared with 42% and 31% for the Belgians and Percherons respectively. This figure compares more favorably with Goulden *et al.* (1985) who determined a prevalence of only 9% in a Clydesdale population. Furthermore, Clydesdale horses did not have a significant association of disease occurrence with height, where as Belgian and Percheron horses did (Brakenhoff *et al.*, 2006), which suggests some degree of breed variation. There is no known reason why this discrepancy exists with Clydesdales, Brakenhoff *et al.* (2006) offered potential reasons

to be associated with sample size and genetic differences among and within each breed.

It is important to check ourselves at this point because RLN is disease of at least four grades and the numbers listed above refer to grades III and IV, which are severe forms of the disease. However, if one looks at the lesser grades of laryngeal paralysis then it is a more generalized distribution of the disease with 80-90% of horses are affected (Cook, 1988). Whilst this piece of work does not stand up to scrutiny, mainly because diagnosis was based on palpation alone, it does generate some provoking thoughts and we should bear that in mind. However, for simplicity's sake, RLN is a disease that affects predominantly young (1-5 years) horses that are tall with long necks but the disease is by no means limited to this conformation of horse.

ii Etiopathogenesis

This has remained a challenging and elusive concept since the disease was first reported over 300 years ago. Once the veterinary world agreed that this disease was in fact the result of disease or damage affecting the nervous supply to the cricoarytenoideus dorsalis muscle and that the "roaring" sound was not a product of mucus secretion the etiology could be sought. Essentially, there are two broad categories, the first is iatrogenic damage to the recurrent laryngeal branch of the vagus nerve. The second is

the more common and is an idiopathic neuropathy hence the alias of idiopathic laryngeal hemiplegia (ILH).

There are a number of specific reports detailing cases where the actual etiology of the RLN could be found and diagnosed, such as:

- Laceration involving the jugular vein resulting in right-sided laryngeal paralysis (Gilbert, 1972).
- Thiamin deficiency resulting in left-sided laryngeal hemiplegia (Loew, 1973).
- Arteriopuncture following catheter placement resulting in right-sided laryngeal hemiplegia (Cramp, unpublished).
- A large abscess (*Staphylococcus aureus*) overlying the caudal larynx and cranial trachea resulted in left-sided laryngeal paralysis (Barber, 1981).
- Abscessation of the cranial mesenteric lymph nodes resulting in left-sided laryngeal hemiplegia (Rigg *et al.*, 1985).
- Retropharyngeal abscessation leading to left-sided laryngeal hemiplegia (Todhunter *et al.*, 1985).
- Guttural pouch mycosis with ipsilateral laryngeal hemiplegia (Church *et al.*, 1986).

- Lead poisoning resulting in laryngeal hemiplegia in foals (Willoughby, 1972)
- Acute laryngeal paralysis was reported in Arabian foals from organophosphate poisoning (Rose, 1978).
- Oral haloxon administration resulting in bilateral laryngeal hemiplegia in Arabian foals (Rose, 1981).
- Perivascular injection of an irritant drug (for example; Phenylbutazone) resulting in left-sided laryngeal hemiplegia (Goulden and Anderson, 1981).
- Bite wound to the neck leading to left-sided laryngeal hemiplegia (Goulden and Anderson, 1981).
- Blunt trauma to the neck from running through a rope fence (Goulden and Anderson, 1981).
- Hepatic dysfunction leading to bilateral laryngeal dysfunction and paresis (Hughes *et al.*, 2009).
- Organophosphate poisoning whilst dosing with contaminated mineral oil led to bilateral laryngeal hemiplegia (Duncan and Brook, 1985).
- Laryngeal dysfunction was noted in 10 out of 11 horses that were diagnosed with Australian stringhalt (Huntingdon *et al.*, 1989).

However, in the majority of cases there is no obvious cause and in these

cases of idiopathic laryngeal hemiplegia there is no established etiopathogenesis. There would almost certainly appear to be a hereditary link and this has been recognized since the early 1900s but more work needs to be done. The very fact that RLN occurs in taller horses and is more prevalent in certain breeds would indicate a familial factor is involved (Cook 1988; Poncet *et al.* 1989; Duncan 1992; Harrison *et al.* 1992). However, familial or not, this still does not explain the etiology of the disease.

One theory is based on a set of mechanical possibilities for why and how this disease occurs and may also go some distance to explaining why this nerve (left) is affected more than the right. The left recurrent laryngeal nerve is approximately 20 cm longer than its counterpart because it runs around the base of the aorta. In 1970, Rooney postulated that the damage to the left recurrent laryngeal nerve was due to the mechanical effects of stretching when the head and neck moved. He suggested that this could cause ischemia and therefore result in nerve damage. However, there are some concerns with this hypothesis, firstly; if tension and stretching does occur, it would have to be greater than 8% of the nerve's existing length before any ischemia can occur (Lundborg, 1988). Furthermore, nerves are relatively robust in the face of ischemic damage and Lundborg (1988) demonstrated that nerves can retain their function for up to 6 hours after total loss of their blood supply. It seems unlikely that even an extreme

postural movement would be maintained for that length of time. This is supported by the fact that Duncan (1987) did not find microscopic lesions that would indicate ischemic nerve injury.

RLN has been characterized histologically and ultrastructurally (Cole 1946; Duncan *et al.* 1978; Cahill and Goulden 1986 (parts I–V), 1987, 1989; Duncan 1987; Duncan and Baker 1987; Duncan and Hammang, 1987; Hahn *et al.*, 2009). The characteristic lesions associated with RLN are characterized by a loss of myelinated fibers that is most profound distally and decreases in amount proximally (Hahn *et al.*, 2009). Histological lesions demonstrate digestion chambers containing axon fragments, axonal atrophy, collapsed myelin sheaths, no axis cylinder and paranodal and internodal accumulations of axonal debris and organelles, which indicates a primary axonal lesion. However, some confusion arises because there is also evidence of extensive myelin damage.

There is some debate as to whether or not RLN is a polyneuropathy or not. The changes are greatest in the distal portions of the left recurrent laryngeal nerves (Duncan *et al.* 1978; Cahill and Goulden 1986a; Hahn *et al.*, 2009), however milder lesions are also present in the right recurrent laryngeal nerve (Duncan *et al.* 1978; Cahill and Goulden 1986a; Hahn *et al.*, 2009). If RLN is a polyneuropathy then it is possible that the myelin damage could be the primary lesion (Hahn *et al.*, 2009), however, if not it is unlikely.

Until the recent work by Hahn *et al.* (2009) this topic had largely be left unchallenged for 15 years. Hahn *et al.* set out to determine whether or not RLN was indeed a mononeuropathy or a polyneuropathy. Horses do not demonstrate clinical signs of polyneuropathy, whereas other species affected by myelinopathies and inherited and metabolic primary axonopathies more typically demonstrate clinical signs affecting multiple nerves. However, there is no evidence of RLN affected horses suffering from megaesophagus, tetraparesis, muscle atrophy and hyporeflexia as we see in dogs with RLN. Interestingly, stringhalt is seen in Australia with RLN (Huntingdon *et al.*, 1987), so perhaps there is a polyneuropathic element in some species or geographical regions. Furthermore, there have been isolated reports of pathologic changes of other long peripheral nerves in RLN cases (Cahill and Goulden 1986c; Hahn and Mayhew, 2007) but these are uncontrolled studies, which do not account for concurrent effects such as toxin-associated disorders. So, there is evidence that it is a polyneuropathy, however it is something that we do not see clinically. The questions that Hahn *et al.* (2009) asked were 1) whether other similarly long nerves have the same pathology and 2) is RLN indeed a polyneuropathy or is it a mononeuropathy?

It is fair to comment that Hahn *et al.* (2009) only used 4 horses and only one of these was a grade IV (complete paralysis) affected patient. They

investigated the distal portions of the median, peroneal and phrenic nerves, which were chosen because they are the longest somatic motor nerves in the horse apart from the recurrent laryngeal nerves. There is no evidence to suggest that these nerves would be the more or less likely affected ones in the case of polyneuropathy. However, their findings were interesting, they stated that RLN is not a polyneuropathy. This is based lack of evidence of axonal lesions in any other long nerves and absence no neurogenic atrophy of the intrinsic musculature of the muscles that these nerves were supplying. Interestingly, they also noted that the right recurrent laryngeal nerve was also significantly affected in RLN cases so the term 'mononeuropathy' should be more correctly applied as a 'bilateral mononeuropathy'. This piece of work by Hahn *et al.* (2009) is the tip of the iceberg and needs to followed up with more in depth studies.

Reference was made to 'neurogenic atrophy', which is the term used to describe the histologic appearance of the muscles affected by RLN. This term represents typical changes, which include atrophied and hypertrophied fibers, centrally placed nuclei, scattered angular fibers, fiber type grouping and increased perifascicular fat (Duncan and Griffiths 1974; Cahill and Goulden 1986d; Duncan *et al.* 1991; Harrison *et al.* 1992). Most commonly, these findings are seen to be more severe in the laryngeal adductor muscle groups, a point that will be discussed further in the next section.

iii. Diagnosis

No diagnosis should ever be attempted without a full and complete history and physical examination. Details surrounding the type of work that the horse is performing, how fit the horse is and at what stage of exercise is the horse suffering. One of the most frequent complaints from owners and riders is that a horse affected with RLN is suffering from poor performance and making a respiratory noise. It is important to get as much detail surrounding the type of noise that is heard. Whilst none of these factors are in themselves diagnostic for RLN they are useful in establishing a sensible list of differential diagnoses.

During a thorough clinical examination, attention should be paid to the cardiovascular and respiratory system, as frequently these horses present for poor performance. Close attention should be paid to the nasal passages, starting at the nares and assessing facial symmetry and looking for signs of nasal discharge and unequal airflows from either nostril. The submandibular lymph nodes should be assessed for enlargement and the jugular grooves and trachea should be palpated for signs of trauma or damage. The trachea and larynx should be auscultated carefully looking for evidence of respiratory disease. The heart and lungs should also be

carefully auscultated to rule out any arrhythmias or primary lower respiratory tract disease as a cause of the poor performance.

Assuming a normal physical examination particular attention should be paid to the larynx. This can be effectively palpated transcutaneously, a diagnosis of RLN can be made by experienced palpators when the muscular process of the arytenoid can be palpated more clearly on one side when compared with the other (Cook, 1988). It is also possible to perform an arytenoid depression test as reported by Marks *et al.* (1970), which consists of producing an axial displacement of the left arytenoid cartilage to produce an inspiratory noise. Another test that can be performed to try and diagnose RLN is the “slap test”. The “slap test” involves slapping the withers on one side and observing or palpating adduction of the contralateral arytenoid cartilage and it tests the integrity of the thoraco-laryngeal reflexes. However, this diagnostic test is unreliable because it is unable to detect reduced adductor function, which is often the first sign of neurologic deficit (Newton-Clarke *et al.*, 1994). This sentiment has been echoed by Hawe *et al.* (2001) who demonstrated that this diagnostic test was not an accurate test for the diagnosis of RLN. So, whilst palpation may not be the answer for definitive diagnosis it is very important as it may allow the examiner to detect other abnormalities such as arytenoid chondritis or the presence of scars indicating previous upper airway surgery (Stick, 2006).

Definitive diagnosis is made via videoendoscopy, this can be done in the standing horse and it can be used to assess the normal structures of the larynx, observe any abnormalities such as chondritis or subepiglottic cysts for example. Because it is a dynamic instrument it can also be used to assess function and movement of the structures of the larynx. Various grading systems for evaluating resting laryngeal function have been established. The system used most consistently grades laryngeal function from I to IV (Rakestraw *et al.*, 1991). This grading system is as follows:

- Grade I: Symmetric and synchronous movement of the arytenoid cartilages.
- Grade II: Symmetric but asynchronous movement of the arytenoid cartilages, abduction maintained.
- Grade III: Asymmetric and asynchronous movement of the arytenoid cartilages, abduction cannot be maintained.
- Grade IV: No perceptible movement of the arytenoid cartilages.

It is important to stress that these grades are used to describe horses that are evaluated at rest. The videoendoscope can be used in horses whilst they

are exercising on the treadmill in an effort to mimic real exercise scenarios, and despite the fact that it is not the same, it has allowed for huge advances in diagnosis of upper airway conditions (Stick & Derksen, 1989; Dart *et al.*, 2001; Durando *et al.*, 2002; Parente *et al.*, 2002; Dart *et al.*, 2005; Tan *et al.*, 2005; Franklin *et al.*, 2006; Lane *et al.*, 2006 (part 1 and 2)). Due to subtle differences that could be seen on treadmill examination of the upper airway tract, subdivision of the grade III affected horses were put in place (Hammer *et al.*, 1998). These subdivisions are as follows:

- Grade IIIA: Asymmetric and asynchronous movement of the arytenoid cartilage but it is able to maintain abduction at exercise.
- Grade IIIB: Asymmetric and asynchronous movement of the arytenoid cartilage but it is unable to maintain abduction at exercise and the affected arytenoid remains in a fixed but incompletely abducted position.
- Grade IIIC: Asymmetric and asynchronous movement of the arytenoid cartilage that leads to severe collapse during exercise.

Subsequently, this scale meant that horses that fell into Grades IIIB, IIIC and IV were surgical candidates.

In addition to palpation, videoendoscopy at rest and at exercise; sound analysis has been used to help diagnose RLN as well as some other diseases of the upper airway (Derksen *et al.*, 2001; Burn & Franklin, 2006). Spectrum sound analysis is used in human medicine in the fields of speech therapy and voice recognition. In 2001, Derksen *et al.* used this software to analyze the respiratory sounds of horses with experimentally induced dorsal displacement of the soft palate and LH. The group identified that inspiratory sounds of horses with LH were characterized by 3 frequency bands (formants). Of particular interest, the second formant, which lay between 0.9 ± 0.06 kHz and 2.4 ± 0.06 kHz (Derksen *et al.*, 2001). The reason that this is of importance is that it lies within the range of human hearing (0.02 to 20 kHz) (Strong & Plintnik, 1992) furthermore; spectrum analysis indicated that the timing, frequency and amplitude of these sounds had an easily recognizable pattern. Not only has this developed into a very useful research tool but it can also be used clinically for horses that do not demonstrate airway obstruction during exercise on the treadmill.

Electromyographical (EMG) analysis has been used to study RLN. It is a technique for evaluating and recording the activation signal of muscles. EMG is performed using an instrument called an electromyograph, to produce a record called an electromyogram. An electromyograph detects the electrical potential generated by muscle cells when they contract, and

also when the cells are at rest. It was first used to study the larynx by Goulden *et al.* (1976), since then it has been used to study a number of muscles in the upper airway (Tessier *et al.*, 2005; Holcombe *et al.*, 2002 and 2007). It has failed to gain widespread acceptance as a diagnostic tool due to its invasive nature and the need for specialist equipment and training.

Ultrasound of the laryngeal structures was first discussed in 1997 but was not really revisited for another nine years other than sporadic case reports. In 2006, Chalmers *et al.* described a technique for ultrasonographic examination of the equine larynx. In this paper the author comments on the limitations of the videoendoscope because it can only see structures from within the lumen of the larynx and thus non-luminal structures including the intrinsic and extrinsic laryngeal muscles and hyoid bones are not evaluated. Therefore although the function of the larynx can be determined, many anatomic components of the larynx and related structures are largely not assessed except by gross palpation. Whilst videoendoscopy is the gold standard for diagnosis of RLN, ultrasonography may provide an adjunctive diagnostic tool. Furthermore, it may provide the general practitioner with a more practical diagnostic aid. Indeed, Chalmers *et al.* (2006) report the diagnosis in one of the cases with grade IV RLN based on the observation that the arytenoid on that side was not moving during ultrasound. In particular reference to RLN, ultrasonography is

useful for the examination of the cricoarytenoideus lateralis muscle (CAL), which is one of the adductor muscles of the larynx. The lateral window provides the best avenue for ultrasound examination when assessing the CAL. The importance of this finding is that the CAL is often first to be affected so if this disease is a bilateral mononeuropathy it should be possible to detect some atrophy on the contralateral side. Whilst still in its infancy, laryngeal ultrasound does show some promise.

The advent of modern imaging modalities, such as magnetic resonance imaging (MRI) and computed tomography (CT), has revolutionized many areas of veterinary diagnostic imaging. In the case of the upper airway their use is usually cost prohibitive if the deterrent of general anesthesia is not sufficient. There is a CT machine for use in the standing horse in the United Kingdom but its use is by no means widespread although the obvious benefits are clearly apparent. So, to date the use of MRI and CT to diagnose upper respiratory tract disease is usually limited to research and isolated case reports. CT has been used to develop a computational flow model of the upper airway at exercise and this has been used to evaluate the degree of abduction of the arytenoid cartilages that is required at various pressures (Rakesh *et al.*, 2008). However, at this stage the use of these imaging modalities is not clinically relevant.

So, what lies ahead? The most tangible of products is the telemetric

endoscope, which allows for endoscopy of the upper airway whilst the horse is exercising in its normal environment (Franklin *et al.*, 2008, Pollock *et al.*, 2009). This is a tremendous advance as it removes some of the valid concerns that are associated with treadmill examination failing to replicate actual exercise conditions. Indeed, we have used our own prototype (Dr. Derksen, unpublished) to diagnose vocal fold collapse in a Belgian mare that could not be viewed with normal videoendoscopic techniques. This is likely to revolutionize diagnostic techniques in the upper airway and it will help elucidate the pathogenesis of the complex disease that is RLN. We eagerly await further data.

iv. *Progression of disease*

This is a difficult and, as yet, unanswered aspect of the disease. An endoscopic survey of 109 Thoroughbreds all under the age of two years old was performed and then repeated 16 months later. At the second examination, 12% of horses had endoscopic examinations consistent with laryngeal hemiplegia suggesting a progression of the disease (Anderson *et al.*, 1997). Another interesting finding from this piece of work was that in 29% of the horses examined, a better grade of laryngeal function was awarded indicating that some horses could recover. More recently, a study looking at older national hunt and sport horses demonstrated that 15% showed progression of disease over a median of 12 months with the onset

of progression occurring at a median age of seven years. In this study there was no sign in improvement in horses with clinical signs (Dixon *et al.*, 2003a). More recently, 197 foals were studied endoscopically and then again one year later, of the 187 foals that were available at this time point only one demonstrated progression to paralysis (0.63%) indicating that RLN was not progressive (Lane JG, 2004). However, this population of very young seems not to represent the more typical clinical population. A series of 3 adult horses with RLN were demonstrated to have progression of the disease using successive dynamic endoscopy (Davidson *et al.*, 2007).

It would appear that whilst RLN can progress, this progression is inconsistent and unpredictable.

4. Treatments for Equine Recurrent Laryngeal Neuropathy

When considering treatment of RLN it is important to remember that this is not a life threatening disease unless it is bilateral in nature. It is a performance limiting disease both in terms of athletic function and also for producing an undesirable clinical sign in the form of noise, which is offensive to the show circuit judges. In the show ring being 'unsound of wind' can result in deduction of points and penalization.

This is an important concept to consider because the decision to perform upper airway surgery should be based on clear evidence that it is needed. As such, each of the procedures that will be talked about below should be targeted to treat a particular problem. Likewise, it is vital that the owner is fully informed of the various advantages and disadvantages of the various procedures prior to performing laryngeal surgery. This relationship between clinician and owner is absolutely vital for a successful outcome. It is important that the correct criteria are used to assess the selection of the procedure, for example, elimination of upper airway noise does not indicate success if restoration of normal upper airway mechanics was the requirement. There is a poor correlation between upper airway noise level and degree of arytenoid abduction as measured by inspiratory pressure, in fact it was found that the greater the abduction the greater the noise produced (Brown *et al.*, 2004). There has also been recognition of the fact that combining some of these procedures is

successful, such as combining ventriculectomy with vocal cordectomy, or ventriculocordectomy with laryngoplasty. The latter of which is commonplace nowadays as both the problems of unwanted noise production and poor performance are addressed.

There is much discussion within the profession as how best to treat RLN. Not only does this discussion revolve around which procedure is best for what horse but also at what stage of the disease one should intervene. It is generally accepted that grade I and II RLN affected horses are clinically normal but that grade III and IV RLN affected horses will require treatment (Stick *et al.*, 2001). Indeed, performing laryngoplasty on grade III RLN affected horses has been suggested as a source of potential failure of the laryngoplasty suture (Hammer *et al.*, 1998). This is suspected to be due to repeated residual contraction of the cricoarytenoideus dorsalis muscle in concert with the adductor muscles of the larynx in horses treated for grade III RLN causing the prosthesis to loosen or cut through the cartilage, resulting in gradual loss of arytenoid abduction or complete laryngoplasty failure due to cyclical loading (Hawkins *et al.*, 1997). Furthermore, the presence of larger CAD muscle mass means that there is more soft tissue for the suture to “cut down” through and thereby loosen to a greater degree (Stick, personal communication). A study demonstrated that eliminating contraction of the cricoarytenoideus dorsalis muscle by performing a recurrent laryngeal neurectomy in conjunction with laryngoplasty and unilateral ventriculocordectomy did not improve the postoperative outcome of Thoroughbred horses treated for left RLN (Davenport-Goodall & Parente, 2003 and Hammer *et al.*,

1998). Interestingly, a recent paper has disposed of the popular concern that grade III RLN-affected horses are a much higher risk of failure suggesting that there is no difference in terms of success if a laryngoplasty is performed on a grade IV RLN affected horse or a grade III RLN affected horse (Witte *et al.*, 2009). Classifying the grade of RLN is not easy. It may be possible to diagnose grades I, II and IV via endoscopy of the standing horse however determining whether or not a horse as grade II or III RLN is more difficult. This demonstrates the need to be thorough when evaluating these patients as well as the need to observe the laryngeal movements at exercise either via a telemetric endoscope or using a treadmill. Furthermore, there may be concurrent dysfunction of other structures in the upper airway confounding the diagnosis of RLN. This point was identified in a study by Lane *et al.* (2006b) where 7 % of horses that had been graded as having normal laryngeal function showed dynamic collapse of the left arytenoid or vocal fold when exercised on the treadmill.

Once the correct diagnosis has been reached, and following sufficient discussion with the owner with regards to their expectations of the horse's requirements, one, or a combination, of the following can be performed.

a. Ventriculectomy

Ventriculectomy has been performed to treat “roarers” for well over 100 years and was made popular by Sir Frederick Hobday in the early to mid 1900s, colloquially becoming known as the “Hobday” procedure (Hall *et al.*, 1990). It refers to the surgical procedure whereby the lateral ventricle (saccule) is removed, it can be performed unilaterally or bilaterally and in combination with any of the other procedures.

Ventriculectomy can be performed via a laryngotomy in the standing sedated horse or under general anesthesia with the horse placed in dorsal recumbency (Stick, 2006; Cramp *et al.*, 2009). It involves the removal of the mucosal lining of the lateral ventricle, which creates a fibrous adhesion between the vocal fold, thyroid and arytenoid cartilages when it heals. This mucosal lining is everted by inserting a “roaring burr” in to the full depth of the ventricle, rotating it to engage the mucosal lining and everting it out of the ventricle. Next, Carmalt forceps can be used to grip the everted mucosa and continue to stretch it out of the saccule where it can be excised using a pair of Metzenbaum scissors. There are reports where the incised edges of the ventricle can be sutured (Tetens *et al.*, 1996) but this is not considered necessary. This adhesion, or scar, may have two effects; one is to make the paralyzed arytenoid cartilage ‘stiffer’ and prevent it from collapsing across the airway under the negative pressure influence of inspiration (Shappell *et al.*, 1988). The second is that it reduces upper airway noise by reducing the turbulence created by the ventricle

when it is left in place (Brown *et al.*, 2003). Typically, the laryngotomy incision will heal within 3-4 weeks and requires basic wound management consisting of cleaning the site 2-3 times per day. The cricothyroid membrane can be closed with absorbable suture for a more aesthetically pleasing finish but this is largely down to surgeon preference (Beroza, 1994 and Boulton *et al.*, 1995). It is recommended based on the work by Brown *et al.* (2004) that horses are rested for 90 days post-operatively, however if the procedure is combined with a laryngoplasty then only 45 days rest is required.

Typically this procedure is performed with a laryngoplasty on the ipsilateral side to reduce the noise associated with laryngeal hemiplegia (Russell & Slone, 1994; Hawkins *et al.*, 1997; Kidd & Slone, 2002; Kraus *et al.*, 2003; Brown *et al.*, 2004; Taylor *et al.*, 2006 and Henderson *et al.*, 2007). However, it can also be performed alone and the effects of ventriculectomy on the equine upper airway were studied by Shappell *et al.* (1988). In this study, the horses had left laryngeal hemiplegia induced by transecting the left recurrent laryngeal nerve and then exercised on a treadmill 30 days after left ventriculectomy. Post-operative inspiratory impedance, airflow, and upper airway pressures were compared to pre-operative values. There was no difference between the two groups, subsequently these horses underwent laryngoplasty and baseline values were restored. Ventriculectomy alone has been successful in treating draft horses with laryngeal hemiplegia (Bohanon *et al.*, 1990). Either way it is generally accepted that ventriculectomy alone should be reserved for

those horses in which the major complaint is that of noise production (Adreani & Parente, 2007).

b. Ventriculocordectomy

This is the same procedure as a ventriculectomy except for the additional removal of the vocal cords. The vocal cords are grasped and a crescent piece of the rostral edge of the vocal cord is resected using a pair of Metzenbaum scissors. Both studies by Tetens *et al.* (1996) and Shappell *et al.* (1988) failed to demonstrate any benefit on upper airway flow mechanics, especially when compared with laryngoplasty. However, limitations to these studies are that they did not evaluate respiratory sound production and the horses were only exercised on the treadmill, which cannot generate enough speed to mimic accurately racehorse performance.

The most convincing work supporting the use of ventriculocordectomy procedures is that carried out by Brown *et al.* (2003). In this experimental study, bilateral ventriculocordectomy reduced indices of upper airway sound to those pre-neurectomy (before laryngeal hemiplegia was induced) after 90 days post-operatively. They also analyzed inspiratory pressure data and discovered that, whilst still higher than pre-neurectomy levels, the inspiratory pressure had significantly decreased by 90 days post ventriculocordectomy. It is interesting to note what the effect of removing the vocal cord really is. A good explanation can be found in the work done by Brown *et al.* (2005). This study compared sound data and inspiratory impedance in

standardbred horses exercised on the treadmill before and after induction of laryngeal hemiplegia following left recurrent laryngeal nerve transection that was treated with laser vocal cordectomy. Their findings indicated that removing the vocal cords improves upper airway flow mechanics to the same degree as ventriculocordectomy but does not reduce noise to the same extent. Improvements in noise reduction were noted but not to the same degree as recorded for ventriculocordectomy. Taken together, these studies would suggest that in order to improve sound data, the ventricles should be removed, however, in order to improve upper airway flow mechanics, albeit not back to baseline values, the vocal cords need to be removed. Consequently, bilateral ventriculocordectomy, which can be performed in the standing sedated patient, or under general anesthesia, with a laser or via a laryngotomy, is the treatment of choice when upper airway noise is the chief complaint.

This statement is further supported by the retrospective data analyzed and reported by Taylor *et al.* (2006), where a unilateral ventriculocordectomy in mild cases of RLN (median grade IIIA) saw 66% of horses make no noise after surgery and 86% of owners were satisfied with the outcome. These data indicate that unilateral ventriculocordectomy is a suitable procedure for horses with low grades of RLN (these included 64 out of 92 National Hunt racehorses) or higher grades of RLN in non-performance horses (Taylor *et al.*, 2006). The obvious advantage of this is that the need for general anesthesia can be avoided with its associated risks as well as avoiding the not insignificant risk of complication associated with the laryngoplasty

procedure (Dixon *et al.*, 2003a; Kraus *et al.*, 2004; Taylor *et al.*, 2006). Nothing is ever that simple, however, with Kraus *et al.* (2003) finding, a few years earlier, that seven out of 104 draft horses that had been treated with ventriculectomy or ventriculocordectomy alone required a laryngoplasty to alleviate the clinical signs. Their conclusion is that ventriculectomy and ventriculocordectomy are insufficient to alleviate the clinical signs RLN in draft horses and that a laryngoplasty should be performed in conjunction for best results. They agree that the risk of general anesthesia in draft breeds is higher than in lighter breeds and that the complications associated with laryngoplasty are much greater compared with ventriculectomy or ventriculocordectomy but they argue that the benefits of laryngoplasty outweigh the risks. This led to a study by Cramp *et al.* (2009) to compare the effects of bilateral ventriculocordectomy with bilateral ventriculectomy on upper airway noise in draft horses. Thirty competitive hitch or pulling draft horses were diagnosed with grade IV RLN via videoendoscopy in the standing horse. Sound data was collected prior to receiving either a bilateral ventriculectomy (n=11) or a bilateral ventriculocordectomy (n=19). All procedures were performed in the standing sedated animal via a laryngotomy incision. Eighty four percent of owners were satisfied with the outcome both in terms of noise production and performance after ventriculocordectomy compared with only 64% of owners satisfied after ventriculectomy. In terms of sound data, both procedures improved indices of upper airway noise (100% of the horses showed improvement) but bilateral ventriculocordectomy improved the inspiratory sound level associated with the second formant, that which is most acutely audible to the human ear (Strong &

Plintnik, 1992). Subsequently, it was concluded that bilateral ventriculectomy and bilateral ventriculocordectomy significantly reduce upper airway noise and indices of airway obstruction in draft horses with RLN, but ventriculocordectomy is more effective than ventriculectomy (Cramp *et al.*, 2009 (in press)).

On the basis of these data, this author concluded that bilateral ventriculocordectomy is the treatment of choice for horses suffering from either milder forms of RLN or severe forms of RLN that do not require a maximum airway for high levels of performance. By reducing the need of laryngoplasty, the morbidity associated with upper airway surgery is reduced as complications associated with ventriculectomy and/or ventriculocordectomy are much less severe and frequent (Dixon *et al.*, 2003a; Kraus *et al.*, 2003; Cramp *et al.*, 2009). It is imperative that a 0.5 to 1 cm length of vocal fold tissue be left at the most ventral aspect, leaving the fornix intact to prevent against laryngeal cicatrix formation, which is a rare but serious complication (Cramp *et al.* 2009).

c. Laser vocal cordectomy, ventriculectomy and ventriculocordectomy

Due to the unsightly and crude appearance of the laryngotomy incision, surgeons have looked to perform these procedures transendoscopically with the use of lasers. The initial work was performed by Shires *et al.* (1990) attempting laser ventriculectomy and whilst the procedure was quick and easy, the results were less than promising with a mucosal remnant being left in the ventricle and thermal

damage of the adjacent structures present. In 2001, Hawkins & Andrews-Jones performed a study evaluating a technique of ventriculocordectomy using a transnasal Nd:YAG laser in a non-contact fashion in 6 horses. The surgical sites had healed by 47 days but there were a small number of complications; latent thermal necrosis of the arytenoid cartilage and mucocoele formation due to small remnants of the ventricle being left behind.

In 2006, Robinson *et al.* reported on the effects of unilateral laser-assisted ventriculocordectomy in horses with laryngeal hemiplegia. Six standardbred horses were used that had laryngeal hemiplegia induced by transection of the left recurrent laryngeal nerve. They were assessed for inspiratory obstruction and respiratory noise post neurectomy and then at 60, 90 and 120 days post-operatively. The surgery was well tolerated and successful, it reduced inspiratory pressures to pre-neurectomy levels but the sound results were less convincing. There was an initial reduction but then the sound level returned to the post neurectomy level indicating no effect. They speculated that this was due to the fact that it was a unilateral procedure, lending further support to the notion of a bilateral ventriculocordectomy should be the treatment of choice. The same group followed up this study by evaluating the histological effects of the procedure (Robinson *et al.*, 2006) and found that it effectively removed the laryngeal ventricle (although care must be taken to ensure that all is removed as some was left in one of the specimens) and that there was no long-term damage to surrounding laryngeal cartilage. An important consideration is that this study was performed with a diode laser, as compared to the Nd:YAG laser,

which has been used in other studies. A diode laser requires less energy than an Nd:YAG laser and therefore there is less risk of latent thermal necrosis of nearby structures.

Henderson *et al.* (2007) followed up this work with a long-term retrospective analysis of the laser assisted ventriculocordectomy technique as described by Robinson *et al.*. Out of 22 horses that had unilateral laser assisted ventriculocordectomy, twenty (91%) horses returned to their intended use. Excessive airway noise was eliminated after surgery in 18 (82%) horses; exercise improved postoperatively in 8 of 10 horses. Three racing Thoroughbreds returned to racing; 1 additional racehorse returned to racing but required a laryngoplasty 1 year later to continue racing. Complications occurred in 3 horses; one had laryngeal swelling, another had exuberant granulation tissue and the third developed right sided arytenoid chondritis, all responded well to further treatment.

So where does this leave us with regard to laser use for ventriculocordectomy? It is a safe and effective procedure so long as the surgeon is careful, uses the correct laser system and everts and ablates the entire ventricle. The technique described by Robinson *et al.* (2006) using a transnasal bur is the most effective but it is more cumbersome and takes more time. The technique described by Hawkins & Andrews-Jones (2001) using a pair of grasping forceps to grab the ventricle is simpler and faster, however, it is likely that some of the ventricle is left in place. At the largest equine hospital in the world, they routinely use this procedure and do not report many

complications, however, it should be noted that the majority of their cases have a laryngoplasty performed as well (Woodie personal communication). How effective this technique would be as a stand alone procedure is still unknown although anecdotal evidence is promising and there is a high level of success.

d. Laryngoplasty

This technique was first described by Marks *et al.* (1970) as an alternative to ventriculectomy and arytenoidectomy, which the authors had determined was ineffective for treatment for athletic horses.

Laryngoplasty aims to replace the function of the atrophied cricoid arytenoideus dorsalis muscle to maintain the “laryngeal lumen in its anatomic position of maximal inspiratory dilatation” Marks *et al.* (1970). Therefore the prosthesis runs from the dorsocaudal edge of the cricoid cartilage to the muscular process of the arytenoid and, when tightened, maintains the muscular process of the arytenoid cartilage in a caudad retraction. Hence it’s colloquial name of the “tieback”. The choice of suture is very much surgeon dependent but large diameter, non-absorbable, monofilament or coated suture material is preferable (Adreani & Parente, 2007). Initially, Marks *et al.* (1970) recommended using a material that had “beneficial elastic properties” and used braided Lycra® but this has since been replaced with more stout materials (Adreani & Parente, 2007).

Horses are placed under general anesthesia in right lateral recumbency with the left side uppermost. The surgical site is routinely prepared and draped, and a 10-12 cm skin incision is made ventral and parallel to the linguofacial vein centered over the palpable edge of the cricoid cartilage. Using careful sharp and blunt dissection, the lateral and dorsal aspects of the larynx are exposed. Retractors are used to maintain exposure whilst the aponeurosis between the cricopharyngeus and thyropharyngeus muscles is transected to expose the muscular process of the arytenoid cartilage. The type of suture and the type of needle is down to surgeon preference, but a large reverse cutting swaged on needle is recommended (this author recommends No. 5 Ticron, Sherwood-Davis & Geck, St. Louis, Mo). The suture is passed from the caudal aspect of the cricoid cartilage, avoiding the lumen of the larynx, and rotated so that it exits the cricoid cartilage 2-3 cm cranial to its caudal border and 1 cm lateral to the dorsal ridge (spine). Next, the needle is removed and the suture is drawn under the muscle belly of the cricopharyngeus muscle. A smaller reverse cutting needle (this author recommends a no.6 Martin uterine reverse cutting needle) is threaded and used to pass through the muscular process of the arytenoid cartilage in a craniolateral direction (Stick 2006). Typically a second suture is placed in the same manner as the first and then they are both tied. An endoscope can be passed so that of the arytenoid cartilage can be observed to assess the degree of abduction, although this is not essential. The thyropharyngeus and cricopharyngeus muscles are sutured together using small diameter monofilament (3-0 PDS) in a simple continuous pattern. The incision is closed in two layers; the first joins the fascia adjacent to the linguofacial

vein to the omohyoideus muscle with small diameter monofilament suture (2-0 PDS) and then the skin is closed in any of the preferred methods.

Since its advent in 1970, the laryngoplasty technique has endured much scrutiny. Arterial blood gas analysis performed before laryngoplasty and 48 hours after the procedure and demonstrated that the hypoxemia and hypercapnia observed pre-operatively after galloping were reversed postoperatively (Bayly *et al.*, 1984). Treadmill experiments demonstrated that experimentally induced laryngeal hemiplegia decreased inspiratory flow and increased inspiratory resistance that could be reversed with laryngoplasty (Derksen *et al.*, 1986). Similarly, in 1988, Shappell *et al.* demonstrated that experimentally induced laryngeal hemiplegia increased inspiratory impedance and trans-upper airway pressure that was reversed following laryngoplasty. The submaximal performance on a treadmill compared with real exercise has always cast some doubt on the usefulness of these studies and in 1990 Williams *et al.* performed two studies to address this. The first looked at racehorses galloping on a track with experimentally induced laryngeal hemiplegia, they measured inspiratory pressures before and after laryngoplasty. They found that whilst laryngoplasty did reduce the pressures it did not reduce them to pre-neurectomy levels. In the second study, horses with clinical (naturally occurring) RLN were evaluated and they were found to have similar increases in upper airway pressures to experimentally induced horses. Furthermore, laryngoplasty did return the upper airway pressures to within the normal range. Tate *et al.* (1993) looked again at blood gas data and acid-base balance of exercising horses with RLN before and after

laryngoplasty. Their data supported that of Bayly *et al.* (1984) nine years previously but also demonstrated that, whilst improved, values did not return to those of normal horses. Again, looking at submaximally exercising horses Tetens *et al.* (1996) found that horses exercising at 75% and 100% of their maximum heart rate demonstrated that the increased inspiratory impedance and decreased flow associated with laryngeal hemiplegia were reversed following laryngoplasty. In conclusion, laryngoplasty can be considered to improve upper airway flow mechanics towards the normal range.

The addition of a ventriculectomy procedure to a laryngoplasty in racehorses does not appear to improve post-operative racing performance compared with laryngoplasty alone (Hawkins *et al.*, 1997). However, if the primary objective is to reduce noise production, or if this is a complaint by the owner then either ventriculectomy or ventriculocordectomy should be performed either unilaterally or bilaterally in addition to the laryngoplasty (Brown *et al.*, 2004).

Most importantly, clinical success needs to be defined. This is difficult to do as reported success rates vary depending the criteria used, which is different for different horses used for different events (Adreani & Parente, 2007). Indeed, Russell & Slone (1994) reported on the success of 70 horses following laryngoplasty and found that 93% of non-racing breeds were improved satisfactorily compared with 48% of racehorses.

If we consider racehorses, it is always difficult to assess racing data as there are so many confounding variables associated with success or apparent lack thereof. However, a number of well-constructed studies have attempted to investigate the success rates of laryngoplasty in racing populations. When 100 thoroughbred racehorses that had laryngoplasty were compared with 400 controls (similar in terms of age, breed, sex and occupation) there was no significant difference between groups (Spiers *et al.*, 1983). Russell & Slone (1994) noted that whilst only 48% of racehorses raced post laryngoplasty, 70% of those less than three years old raced compared with only 25% of those horses older than three years. A large retrospective study of 174 thoroughbred racehorses and 56 standardbred racehorses sought to evaluate success with relation to a number of variables such as breed (Thoroughbred v Standardbred), endoscopic grade of laryngeal function, ventriculectomy versus no ventriculectomy, type of prosthetic suture used, and number of prostheses placed (Hawkins *et al.*, 1997). The findings of this study were that laryngoplasty with or without ventriculectomy allowed 77% of the horses to race at least one time after surgery, improved racing performance in 56% of the horses that completed three races before and after surgery, and improved subjectively evaluated racing performance in 69% of the horses. In 2000, Strand *et al.* used a statistical method to evaluate racing performance of inexperienced and experienced groups of racehorses after laryngoplasty with bilateral ventriculectomy. Their data demonstrated that approximately 60% of all horses won one race after surgery and that 25% won at least three races after surgery, indicating that laryngoplasty is a successful procedure. However, experienced horses never returned to their baseline levels, in other words,

they were improved by the surgery but not returned as good as new. Therefore, their conclusion was that “it may be prudent to provide a guarded prognosis for full restoration of racing performance in older horses, unless they are especially talented and are free of other racing-related problems” (Strand *et al.*, 2000).

There has always been some controversy over whether or not horses with grade III RLN respond as well as horses with grade IV RLN (Hawkins *et al.*, 1997). In 2001, Davenport *et al.* set out to investigate the effects of recurrent laryngeal neurectomy in combination with laryngoplasty and ventriculocordectomy on the postoperative performance of Thoroughbred race-horses treated for grade III left laryngeal hemiparesis. Fifty-five horses with grade III RLN as diagnosed via videoendoscopy in the standing horse were treated with laryngoplasty and unilateral ipsilateral laser assisted ventriculocordectomy, 39 had a recurrent laryngeal neurectomy performed at the same time and 16 did not. The idea behind this is that laryngoplasty in grade III RLN affected horses is less successful than in grade IV RLN affected horses due to residual muscle movement that cycles the suture more quickly, the fact that the cricoarytenoideus dorsalis muscle atrophies and loosens the laryngoplasty or that the suture cuts through the larger mass of muscle becoming more loose. Davenport *et al.* (2001) performed the neurectomy so that there would be no cycling of the muscle, however, there was no benefit to neurectomy suggesting that cycling has no effect. Witte *et al.* (2008) recently demonstrated that horses treated by laryngoplasty and unilateral laser ventriculocordectomy for grade III RLN performed better than those with grade IV RLN. It would appear that there is no evidence that laryngoplasty is

doomed in horses with grade III RLN and is still recommended as an appropriate treatment by many surgeons.

In non-racing horses, the results of laryngoplasty are unsurprisingly reported to be better than for racehorses (Adreani & Parente, 2007). In 2003, Dixon *et al.* performed a large study on 200 mixed breed horses that had laryngoplasty performed and 86% of owners considered the procedure to have been worthwhile. In the same study, 91% of were able to resume training six weeks postoperatively. This group also noted that when noise reduction was the major barometer for success, and when combined with ventriculocordectomy, 73% were considered to make no abnormal respiratory noise postoperatively. This compares well with the 73% of draft horses where noise was eliminated following laryngoplasty with ventriculocordectomy (Kraus *et al.* 2003). These percentages are markedly higher than the 25-47% range quoted for racehorses (Russell & Slone (1994) and Hawkins *et al.* (1997)). It would appear from these data that laryngoplasty is a very successful procedure in non-racehorses probably because these horses do not require the full arytenoid abduction necessitated by the athletic endeavours of a racehorse. In fact, the more abduction achieved, the higher the risk of postoperative complications, which begs the question: Is it worthwhile performing a laryngoplasty on non-racehorses at all?

Post-operative care consists of stall rest for 30 days with handwalking only, then two weeks of small paddock turnout or light exercise. After 45 days, training can be

resumed. Recommendations for diet are limited to feeding hay from the ground to minimize post-operative coughing (Hawkins *et al.*, 1997; Dixon *et al.*, 2003a).

i. Postoperative Complications of Laryngoplasty

The complications associated with this procedure have always been the disadvantage of the procedure. A balance needs to be found between the performance benefits of laryngoplasty and the risk of serious complications developing. This is the main reason why there is a substantial amount of continued research into finding alternative and 'better' treatment options than laryngoplasty.

Coughing is the most likely postoperative complication. This results from a degree of dysphagia and the greater the degree of arytenoid abduction is significantly associated with postoperative coughing (Russell & Slone (1994) and Dixon *et al.* (2003a)). During deglutition the arytenoid cartilages adduct in concert with the vocal cords. This movement combined with the dorsal position of the epiglottis protect the upper airway from being contaminated. However, if a prosthesis is preventing the left arytenoid from adducting, or worse still, this prosthesis is maintaining the arytenoid in a position of exaggerated abduction, this protective function will not be possible. The larynx and carina of the trachea are highly sensitive to light touch or small amounts of foreign material (Guyton & Hall, 2006). If stimulated, afferent nerve impulses pass via the vagus nerve to the brain and initiate a series of autonomic sequences resulting in a cough where by the

unwanted material is rapidly exhaled. Furthermore, there is some speculation that damage to the cranial laryngeal nerve during surgery (during dissection between the cricopharyngeus and thyropharyngeus muscles) may result in disruption to the pharyngeal constrictor muscles thereby interrupting the swallowing reflex permanently, a separate issue to that of the abducted arytenoid physically obstructing the esophagus (Greet, 1979). The incidence of post-operative coughing has been reported to be as high as 43% (Dixon *et al.*, 2003a). It is not uncommon for coughing to be present in the initial postoperative period but it should resolve in a matter of days to weeks. In the above paper, the 43% referred to coughing for less than 6 months, 24% of which coughed when eating and 19% had no association with eating. Fourteen percent of these horses had a cough, which persisted for longer than 6 months (Dixon *et al.*, 2003a). The chronic cough can be more of an irritation to the owner and horse in some cases (approximately half of the these reported by Dixon *et al.*). However, in some, it can develop into pulmonary disease and occasionally into life threatening aspiration pneumonia. Thankfully, the number of cases that develop fulminant bacterial pulmonary infection is few (Dixon *et al.*, 2003a) especially when one considers that 3-10% of horses have endoscopic evidence of food in the trachea postoperatively (Russell & Slone (1994) and Strand *et al.*, 2000).

As with any permanent implant, the prosthesis and the wound can become infected. Since its advent in 1970, the prosthesis was performed using braided Lycra®, which was associated with a high rate of infection, up to 15% being

reported in the literature (Adreani & Parente, 2007). Since then monofilament or coated braided multifilament suture materials have been used and this percentage has decreased. Currently, the reported incidence of wound infection is between 0.5 and 6% (Hawkins *et al.*, 1997; Strand *et al.*, 2000; Davenport *et al.*, 2001, Kidd & Slone, 2002; Dixon *et al.*, 2003a; Kraus *et al.*, 2003). However, whilst this range is not dissimilar for any elective, clean surgical wound the frequent presence of a laryngotomy incision increases the risk (Dixon *et al.*, 2003a). The incidence of postoperative swelling associated with the laryngoplasty wound is 17% (Dixon *et al.*, 2003a), however more than half of these resolved within a couple of weeks. Despite the fact that most of the of the incisional infections resolve by themselves, meticulous wound care of the laryngotomy incision, if present, is vital and careful hemostasis and dissection around lymph vessels is important during surgery. Suture penetration into the airway, through the airway mucosa, can result in inflammation and infection. This is best avoided by intraoperative endoscopy so that the suture can be replaced at the time of surgery. A far less common complication is that of granuloma formation and chondritis, which occur in 1% of (Dixon *et al.*, 2003a).

The last major complication associated with this procedure is loss of arytenoid abduction. This phenomenon occurs in every case to some degree (Dixon *et al.*, 2003a), however, it results in failure of the procedure in 2-15% of cases (Marks *et al.*, 1970; Hawkins *et al.*, 1997; Dixon *et al.*, 2003a; Kraus *et al.*, 2003). In these cases where failure of the prosthesis has resulted in total loss of arytenoid

abduction, it is usually the result of failure of the cartilage at the muscular process of the arytenoid cartilage (Dean *et al.*, 1990; Dixon *et al.*, 2003a). A number of different prosthetic materials and techniques have been tested and whilst some have demonstrated increased strength none have solved the issue of cartilage failure (Dean *et al.*, 1990, Scherzer *et al.*, 2005 and Rossignol *et al.*, 2006). One of the most promising studies was that of Schumacher *et al.* in 2000. This group performed an *in vitro* study investigating properties of a prosthesis that is composed of a steel cable and stress-reducing washers. Whilst the *in vitro* leg of the study provided encouraging results, it has failed to gain widespread acceptance clinically due to its impractical technical application.

In a computational flow model study investigating decreasing degrees of abduction of the left arytenoid cartilage it was noted that as the left arytenoid lost abduction different flow of air through the larynx was encountered (Rakesh *et al.*, 2008). They found that when the left arytenoid had relaxed to the grade 3 position (Dixon *et al.*, 2003b) there was increased airflow indicating collapse associated with the right vocal cord and aryepiglottic fold. Subsequently, a recommendation to remove the right aryepiglottic fold in concert with a laryngoplasty was made in the discussion section of this paper. This raises the question of which additional procedures should be performed along with a laryngoplasty. Laryngoplasty will return upper airway mechanics to normal levels and therefore should improve the performance of the horse, however, without an additional procedure upper airway noise will still be produced. Removal of the ipsilateral vocal cord and lateral

ventricle should reduce this noise, however, if the right vocal cord is also involved as indicated and we know that the lateral ventricles cause noise then both of these should also be removed. So, the recommendation would be bilateral ventriculocordectomy with a laryngoplasty. According to the work by Rakesh *et al.*, 2008 we should also consider the removal of the right aryepiglottic fold, however, one wonders whether their results would have been the same if the laryngoplasty had been performed with any other procedure at the same time? These issues still warrant further investigation.

e. Partial Arytenoidectomy

Typically this technique is more commonly performed to treat unilateral or bilateral arytenoid chondritis. However, it has also been used to treat RLN and whilst there are many forms of arytenoidectomy, partial arytenoidectomy has been shown to reduce postoperative obstruction the most (Belknap *et al.*, 1990; Williams *et al.*, 1990; Lumsden *et al.*, 1994, Radcliffe *et al.*, 2006).

The technique is performed via a ventral laryngotomy incision with the horse in dorsal recumbency. It is necessary to place the endotracheal tube through a tracheotomy incision so that the surgeon can work freely on the larynx. The corniculate process of the arytenoid is removed en bloc with its mucosa. Next, the arytenoid cartilage is exposed by dissecting away the overlying mucosa using a scalpel blade. The incision is made along the caudal border of the laminar portion of

the arytenoid from the dorsal midline ventrad and then continued craniad along its ventral border. The mucosa is carefully elevated using a periosteal elevator and the now exposed laminar portion of the arytenoid cartilage is freed from its deep muscular attachments. The loose arytenoid cartilage is dissected free from the muscular process via the articulation. Next a ventriculocordectomy is performed in the standard manner and the mucosal flap is closed using a simple continuous pattern of absorbable suture material (Stick, 2006). Primary closure of the mucosa is debated but it is recommended in order to reduce the risk of developing excessive postoperative granulation tissue (Parente *et al.*, 2008). However Barnes *et al.* (2004) argue that the procedure is as effective without closure of the mucosa and that this is advantageous due to the reduced surgical time and avoidance of the associated complications such as suture line dehiscence, hematoma formation, thickening and fibrosis. To close the mucosa or not to close the mucosa likely comes down to surgeon preference and it is this author's recommendation to close the mucosa in an effort to speed healing and make the airway as smooth as possible.

More recently, partial arytenoidectomy has been investigated for the treatment of RLN as well as arytenoid chondritis (Barnes *et al.*, 2004; Radcliffe *et al.*, 2006; Parente *et al.*, 2008; Witte *et al.*, 2009). Barnes *et al.*, 2004 reported that partial arytenoidectomy without mucosal closure would return 61-78% of racehorses to successful racing. They stated that these horses had a fair prognosis and their results were much better than the previously reported results of 45% (Tulleners *et al.*, 1988). They also reported no complications associated with the surgery, however, only 55%

of those horses raced three or more times implying some degree of exercise intolerance (Parente *et al.*, 2008). In 2006, Radcliffe *et al.* compared laryngoplasty and unilateral ventriculocordecotomy to partial arytenoid in experimental horses exercising submaximally and at maximum heart rate. They found that at submaximal speeds there was no difference between the two procedures but that at maximum heart rate laryngoplasty demonstrated a slight improvement. It should be noted that the treadmill does not perfectly mimic natural exercise and that the partial arytenoidectomy was performed on the same animal after the laryngoplasty and ventriculocordecotomy and there was likely some effect of the first procedure superimposed on the results. It is also possible that the low power of the study makes the difference between the two procedures less obvious. However, partial arytenoidectomy can yield very positive results, 82% of horses that had partial arytenoidectomy raced after surgery and 64% raced more than five times (Parente *et al.*, 2008). Not all of these horses were being treated for RLN, 22 out of the 76 were treated for failed laryngoplasty, the rest for arytenoid chondropathies, however, the procedure still shows some promise. It should be noted that 17% of these horses have a second procedure to laser debride via an endoscope excessive granulation tissue at the surgery site despite primary mucosa closure in all of the cases. The median time from surgery to racing was six months, which may be considered a lengthy lay off but these cases are repeat procedures due to a failed first attempt. The notion that partial arytenoidectomy is a successful procedure with a favorable prognosis for racing is supported by a recent study by Witte *et al.*, 2009 comparing partial arytenoidectomy with laryngoplasty and ipsilateral ventriculocordecotomy. They suggest that a partial

arytenoidectomy may be less technically demanding than a laryngoplasty and because there is no prosthesis it may be an attractive alternative to the laryngoplasty if the outcome is no different. Furthermore, it may be a preferable procedure in grade III RLN cases where surgeons are concerned about the anecdotal notion of cyclical fatigue and failure due to residual movement of the arytenoid, or loosening of the suture due to increased muscle mass and subsequent atrophy and “cut down” of the suture material (Ducharme & Hackett, 1991; White 1992). This study demonstrated that horses with grade III RLN treated with laryngoplasty and ipsilateral ventriculocorpectomy showed postoperative earnings comparable to controls and was actually better than those horses with grade IV RLN. This dispaches the theory that grade III RLN should be treated with a partial arytenoidectomy because a laryngoplasty procedure is more likely to fail (Witte *et al.*, 2009). This group also noted that whilst both procedures lead to similar rates of return to racing, laryngoplasty with ipsilateral ventriculocorpectomy benefits from higher postoperative earnings and therefore should still be recommended as the treatment of choice (Whitte *et al.*, 2009).

Partial arytenoidectomy should be used as a secondary procedure to treat those horses where laryngoplasty has failed and in these instances it can be recommended with a good degree of confidence. It should not be used as a first line of treatment due to the complications associated with the procedure, furthermore, it should not be recommended for those horses where noise reduction is the primary goal of the procedure. Postoperative noise production frequently occurs following partial

arytenoidectomy due to residual mucosa including the aryepiglottic fold that is no longer held in place by the corniculate process of the arytenoid cartilage (Adreani & Parente, 2007). Parente *et al.*, 2008 identified the need to endoscopically examine patients one month post partial arytenoidectomy due to the 17% likelihood of developing granulation tissue, which can be easily debrided via the endoscope using a laser on an outpatient basis. However, the most serious complications associated with partial arytenoidectomy are those of dysphagia and aspiration resulting in coughing and potential pneumonia. The biggest disadvantage of this procedure and why another procedure should be performed first in cases of RLN is that it is irreversible. If a laryngoplasty results in dysphagia, the prosthesis can be loosened or removed but if an arytenoidectomy results in dysphagia and coughing there is nothing that can be done other than management practices to resolve this.

Postoperative management consists of broad-spectrum antibiotics for 3-5 days and anti-inflammatories for 7-10 days as well as feeding from the ground with soaked hay until horses can demonstrate that they can eat normally without coughing. Horses should be stall rested for 4-6 weeks prior to complete healing, which usually occurs within 8 to 10 weeks (Stick, 2006).

f. Nerve Muscle Pedicle Graft (NMPG)

This procedure consists of implanting nerve muscle pedicles from the omohyoideus muscle (the first cervical nerve) and implanting them into the affected CAD muscle..

The main advantage to laryngeal reinnervation using a nerve muscle pedicle graft is the preservation of the normal architecture of the larynx and thereby minimizing postoperative complications compared with other techniques such as laryngoplasty and partial arytenoidectomy (Fulton, 2007). Typically, this author performs a bilateral ventriculocordectomy in the standing sedated horse a number of days after the NMPG. The reason for this additional procedure is two-fold; firstly, it is performed to reduce the noise production satisfactorily for the owner. Secondly, it is performed to improve performance especially in the non-racing breeds. Whilst evidence of reinnervation is noted after 3-4 months postoperatively, complete return to function may take up to 12 months. However, useful endeavor by the horse may be possible within this time frame with a bilateral ventriculocordectomy resulting in greater owner satisfaction.

The postoperative management is very important and very specific to this procedure. The following is taken from Fulton, 2003 and this regime was based on experiments performed on dogs, humans and horses (Hengerer & Tucker, 1973; Tucker, 1976; Fulton, 2001). Postoperatively, the horses are confined to a stall for a period of 2 weeks. A neck bandage should be placed at surgery and horses should be in stalls where they cannot put their head over the door and rub the neck incision. The bandage can be removed 2-3 days postoperatively and sutures are removed after 14 days. The prophylactic antibiotics penicillin and gentamicin are routinely administered for 3-5 days. Anti-inflammatory medication is administered for 7-10 days after surgery (Fulton *et al.*, 2003).

After stall confinement, a further period of 2 weeks in a small yard or paddock followed by normal paddock turnout for 12 weeks (Fulton *et al.*, 2003). At this stage the horse should go into training (16 weeks after surgery, 12 weeks is the earliest time that reinnervation has been seen). When the horses are returned to exercise, it is advised that episodes of fast exercise are introduced as early and as frequently as possible. The omohyoideus muscle is an accessory muscle of respiration, considerable respiratory effort must be undertaken to activate the first cervical nerve. Therefore, it is recommended that Thoroughbreds gallop over 400 m every second day of training (Fulton *et al.*, 2003).

After 6 weeks of training, trainers/owners should present the horse for endoscopic assessment of the larynx. At rest, the left arytenoid cartilage most commonly looks exactly as it did before surgery because the omohyoideus is not functioning at rest and therefore the first cervical nerve is not stimulated. There are two diagnostic reflexes that can be performed, one involves stretching the horse's head and neck upward and if reinnervation has occurred, there is brief abduction of the left arytenoid cartilage. The second consists pulling back sharply on the commissure of the horse's lips resulting in a sudden and brief abduction of the left arytenoid cartilage. If reinnervation has occurred then the trainer is to continue training toward a return to racing. If there is no evidence of reinnervation the horse should be turned out again for another 8 weeks and repeat the same exercise and series of evaluation procedures. At the second visit a period of approximately 9 months has elapsed since surgery and

if there is no improvement then clinical experience suggests that it is unlikely (Fulton *et al.*, 2003). However, Fulton comments that horses can take up to 12 months to show evidence of successful reinnervation (Fulton *et al.*, 1991).

The only real report of results is from Fulton *et al.*, 2003 and again from Fulton, 2007 as a follow up with more horses having been studied. Fulton, 2007 reports that 76% of unraced horses and 84% of raced horses demonstrated evidence of reinnervation. Of these reinnervated racehorses 54% won one or more races after surgery, 58% had overall improved performance rankings after surgery, 51% had higher overall prize money after surgery while 57% had more money per start after surgery. Of the reinnervated unraced horses 66% started in one race, of these 63% won at least one race. Out of 11 standardbred horses that have undergone nerve muscle pedicle graft surgery six have raced, three were retired with unrelated problems, one died and one was a failure (Fulton, 2007). Three out of the six horses earned more money after surgery (but raced in more races) and five out of six owners were satisfied with the procedure. Seven warmbloods have been reported on and five have performed at a higher level postoperatively, which compares more favorably than the 57% success rate that Tyler, 2000 reported. The procedure compares favorably with laryngoplasty but suffers due to the long recovery time.

The postoperative complications are limited to seroma, hematoma and infection of the surgical site but in general complications occur infrequently. Three out of 182 horses

that have demonstrated successful reinnervation that later became denervated for unknown reasons (Fulton, 2007).

This is a promising technique, however, the limiting factor is the time it takes for reinnervation to result in the cricoid arytenoideus dorsalis muscle being able to perform its function. This is in part due to the process of reinnervation and in part due to the mechanical effort that the cricoid arytenoideus dorsalis muscle has to overcome to abduct the arytenoid. So far we have identified the need for a superior technique to laryngoplasty due to the high complication rate associated with the procedure. We have also identified a procedure (NMPG) that has a similar success rate and avoids the serious complications associated with laryngoplasty. However, as aforementioned, the time period taken for return to function is unattractive to owners and trainers alike and this is a severe limitation to the NMPG. The rest of this Thesis looks at two attempts to shorten this convalescence period. The first study investigates the effect of angle and force with regards to laryngeal abduction in an effort to determine whether the medial or lateral muscle belly of the CAD muscle are more important in laryngeal abduction. NMPGs placed in a location that confers significant mechanical advantage may be able to return the CAD to its appropriate function sooner. The second study investigates the effect of electrostimulation of the implanted first cervical nerve in an effort to improve reinnervation specifically and to produce muscular hypertrophy of the CAD. The aim is to be able to shorten the recovery time of the NMPG procedure so that it is competitive with the current gold standard (laryngoplasty).

5. Effect of magnitude and direction of force on laryngeal abduction: Implications for the nerve-muscle pedicle graft technique

a. Introduction and Aims

By way of reminder, the nerve-muscle pedicle graft procedure needs to be modified and improved before there will be widespread acceptance. When performing a NMP graft, three to 4 pedicles are usually available for transplantation, but the optimal location for placement of the pedicles within the CAD muscle is currently unknown. Recently, an elegant study described the neuroanatomy of the equine CAD muscle (Cheetham *et al.* 2008). There is a medial and lateral neuromuscular compartment and the lateral compartment plays a greater role in abduction of the arytenoid cartilage. Therefore, optimal placement of neuromuscular pedicle grafts may improve surgical efficacy, including improved laryngeal abduction and shortening of the recovery time.

The aim of this study was to determine force-abduction relationships at various lines of action on the muscular process of the arytenoid cartilage. The medium-term goal was to assist surgeons in choosing the optimal placement of NMPGs.

The hypothesis of this study was that the magnitude and direction of force placed on the muscular process of the left arytenoid cartilage affects the magnitude of laryngeal abduction.

b. Materials and Methods

Five larynges were collected from horses that had been euthanased for reasons unrelated to upper airway disease. This study was approved by the All-University Committee on Animal Use and Care at Michigan State University.

Specimen preparation

Immediately after euthanasia, the larynges were removed from the cadavers by sharp dissection. Visual inspection of the larynges failed to reveal any abnormalities. The pharyngeal musculature was dissected from each of the specimens prior to storage at 0°C in a 2% solution of 2-phenoxyethanol, a solution known to preserve tissue pliability (Lussier *et al.* 1996). Specimens were tested within 24 hours of harvesting and were allowed to return to room temperature before each experiment. Larynges were placed into a custom-made jig and were secured using a polymer resin¹. Furthermore, 1 screw was placed vertically through the epiglottis, and a second screw was placed through the centre of the cricoid cartilage into the jig. To tie back the right arytenoid cartilage in full abduction, a # 5 Ticon² suture was placed through the

¹ Hydroplastic, TAK Systems 20 Kendrick Rd. Wareham, MA 02571

² Syneture, United States Surgical, Customer Service, 150 Glover Avenue, Norwalk, CT 06856

caudal-most aspect of the cricoid cartilage and through the muscular process of the right arytenoid cartilage, and secured under maximal tension. With the right arytenoid cartilage 'tied back' maximally, another length of # 5 Ticron suture was secured to the muscular process of the left arytenoid cartilage and attached via a mechanical force transducer³ to a dead-weight force generator. Biomechanical markers were placed on the rostral face of the rima glottis at the ventral aspects of the left and right corniculate processes (points A and B, respectively); halfway between the most dorsal and ventral aspects of the left corniculate process (point C); and at the 2 points where the left and right aryepiglottic folds join the epiglottis (points D and E, respectively) (Figure 1). A digital camera was mounted on a tripod and was positioned so that its view was orthogonal to the plane of the rima glottis.

Experimental protocol

Using increments of 0.98 N, the force generator applied a force of 0 N through 14.70 N for 1 minute each to the left muscular process at 0, 10, 20, 30, 40, 50, 60, and 70 degree angles (Figure 2). The 14.70 N endpoint was chosen because in preliminary experiments visual inspection revealed that additional force did not further abduct the left arytenoid cartilage, and because this force is significantly less than the 55.8 - 219.6 N force required to cause failure of the laryngoplasty at the muscular process (Schumacher *et al.*, 2000). Zero degrees was defined as a line through the muscular process of the left arytenoid cartilage and parallel to the wing of the thyroid cartilage. The sequence of angles studied was randomized by use of a random numbers table.

³ Transducer Techniques Inc., CA, USA

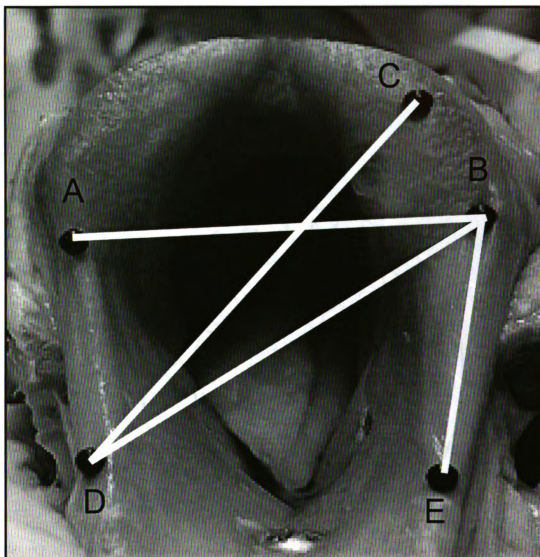


Figure 1: Rostral view of larynx showing biomarkers used to assess degree of left arytenoid abduction.

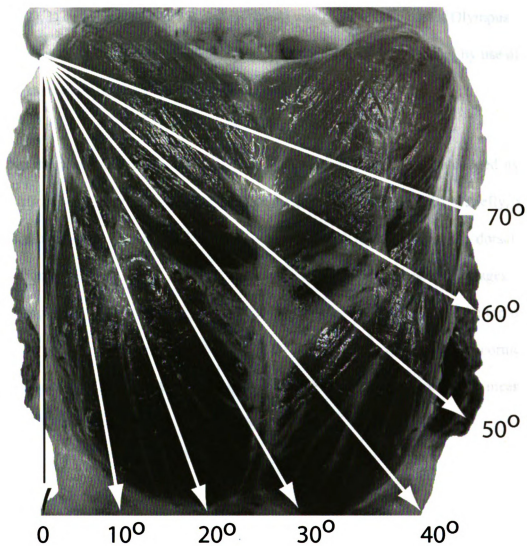


Figure 2: Dorsal view of larynx showing direction of force (0-70 degrees) placed on the muscular process of the left arytenoid cartilage.

A digital image was captured of the rima glottis one minute after each force had been applied. In each digital image the distance between points A and B (line 1), D and C (line 2), D and B (line 3), and B and E (line 4) was measured by use of Olympus Biological Suite software⁴. For each larynx, the software was calibrated by use of a ruler that was incorporated into the first digital photograph.

From the photographs, the right to left angle quotient (RLQ) was calculated using a modification of a previously described method (Jansson *et al.* 2000). Briefly, a line was drawn from the ventral-most aspect of the vocal fold fornix to the dorsal-most junction of the left and right corniculate processes of the arytenoid cartilages. This line was then extended dorsad by one third of its original length. From the top of this line, tangential lines were drawn to the dorsal aspects of the left and right corniculate processes. The angles between the dorsoventral line and the tangents were measured. The RLQ was calculated by dividing the right angle by the left angle.

Statistical analysis

Data were analyzed by three-factor ANOVA with the fixed effects of tension and angle and the random effect of horse. Differences between the means were determined by post-hoc Tukey comparisons. The level of significance was set at $P \leq 0.05$.

⁴ SIS MicroSuite5 Biological Software, Olympus America, Inc., Center Valley, Pennsylvania

c. Results

Increasing force from 0 N to 14.70 N progressively and significantly increased the length of lines 1-4 (Figures 3a-d) and RLQ (Figures 4 and 5) ($P < 0.001$). Furthermore, there was a significant interaction between force and angles (line 1 [Figure 3a] $P < 0.016$; lines 2-4 [Figures 3b-d] and RLQ [Figures 4 and 5] $P < 0.001$). Differences in the amount of abduction of the left arytenoid cartilage caused by angle became significant at a force of 7.84 N for lines 2 (Figure 3b), 3 (Figure 3c), and 4 (Figure 3d), and RLQ (Figure 4), and at a force of 11.76 N for line 1 (Figure 3a). At 14.70 N mean abduction achieved at angles 10° or 30° was always greatest while abduction at angle 70° was always least. At this force the differences between angles 10° or 30° and angle 70° were significant for all indices of abduction, whilst differences between angles 0 through 30, and angles 40 through 70 were not significant.

d. Discussion and Conclusions

While the NMP graft technique used in the treatment of RLN has been described in detail, little attention has been paid to the optimal placement of the NMPs within the CAD muscle. Reinnervation of the CAD muscle emanates from the grafted NMPs, and occurs gradually. Results of the current study demonstrate that direction of force applied to the muscular process of the arytenoid cartilage is an important factor in

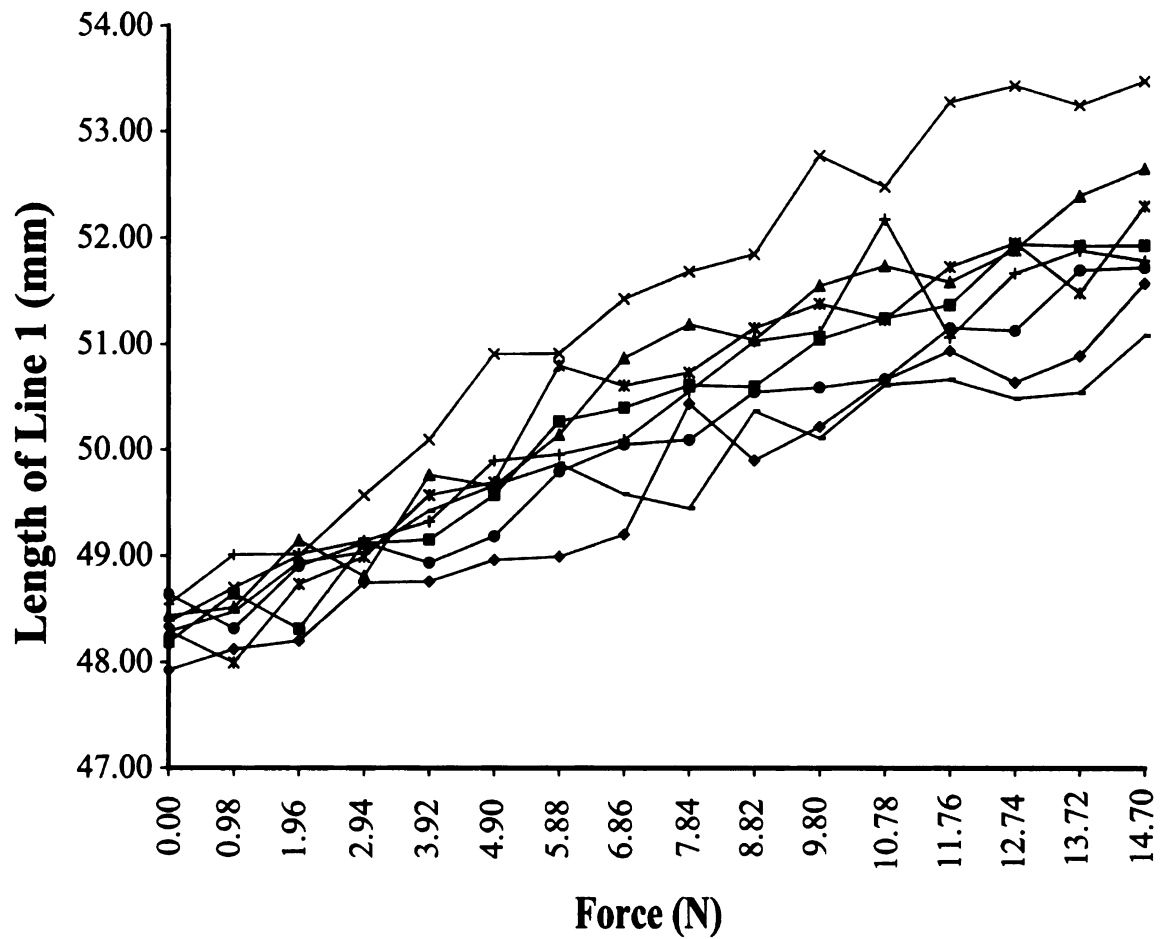


Figure 3a: Effect of force (Newtons) and angle (degrees) on the length of Lines 1 (a), 2 (b), 3 (c), and 4 (d). ◆ 0 degrees; ■ 10 degrees; ▲ 20 degrees; × 30 degrees; * 40 degrees; ● 50 degrees; + 60 degrees; and - 70 degrees.

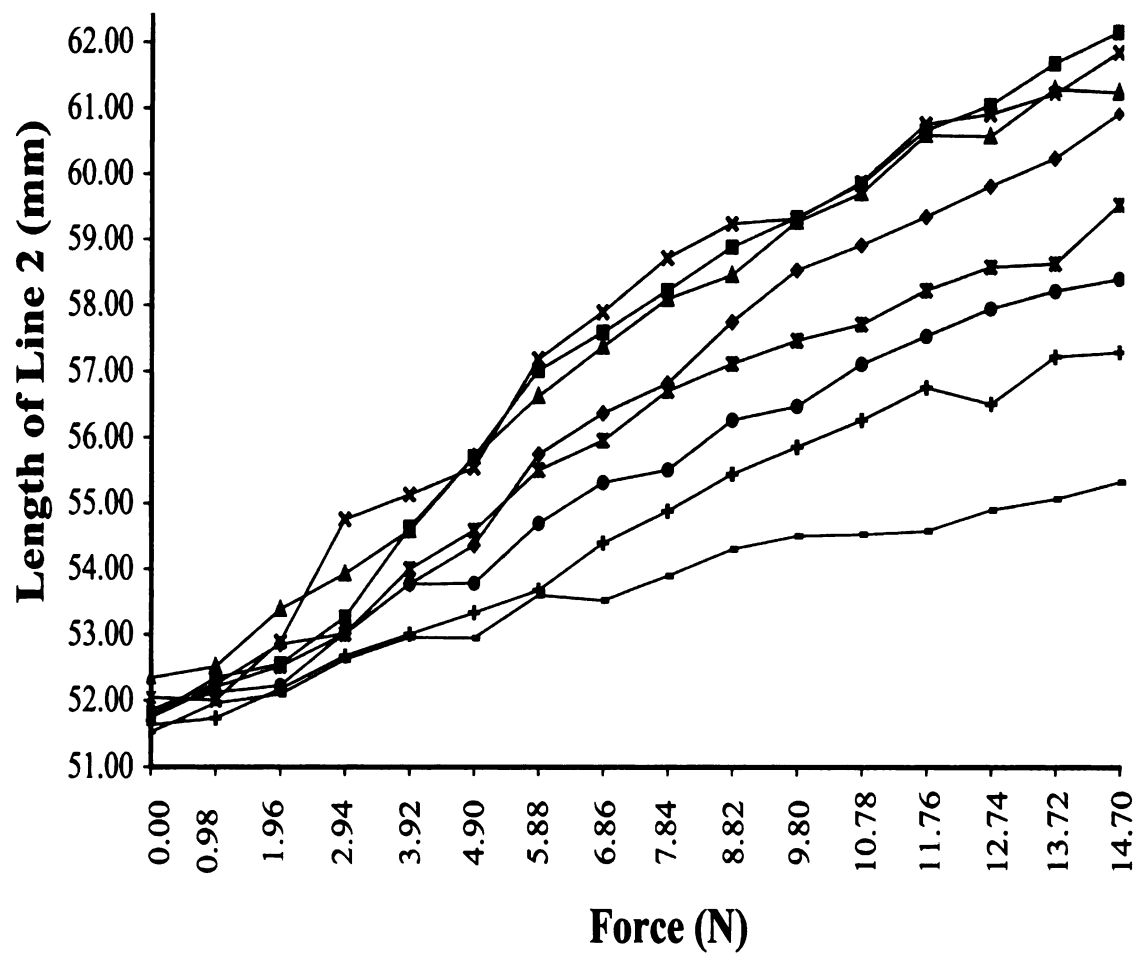


Figure 3b: Effect of force (Newtons) and angle (degrees) on the length of Line 2.
 ◆ 0 degrees; ■ 10 degrees; ▲ 20 degrees; × 30 degrees; * 40 degrees; ● 50 degrees; + 60 degrees; and – 70 degrees.

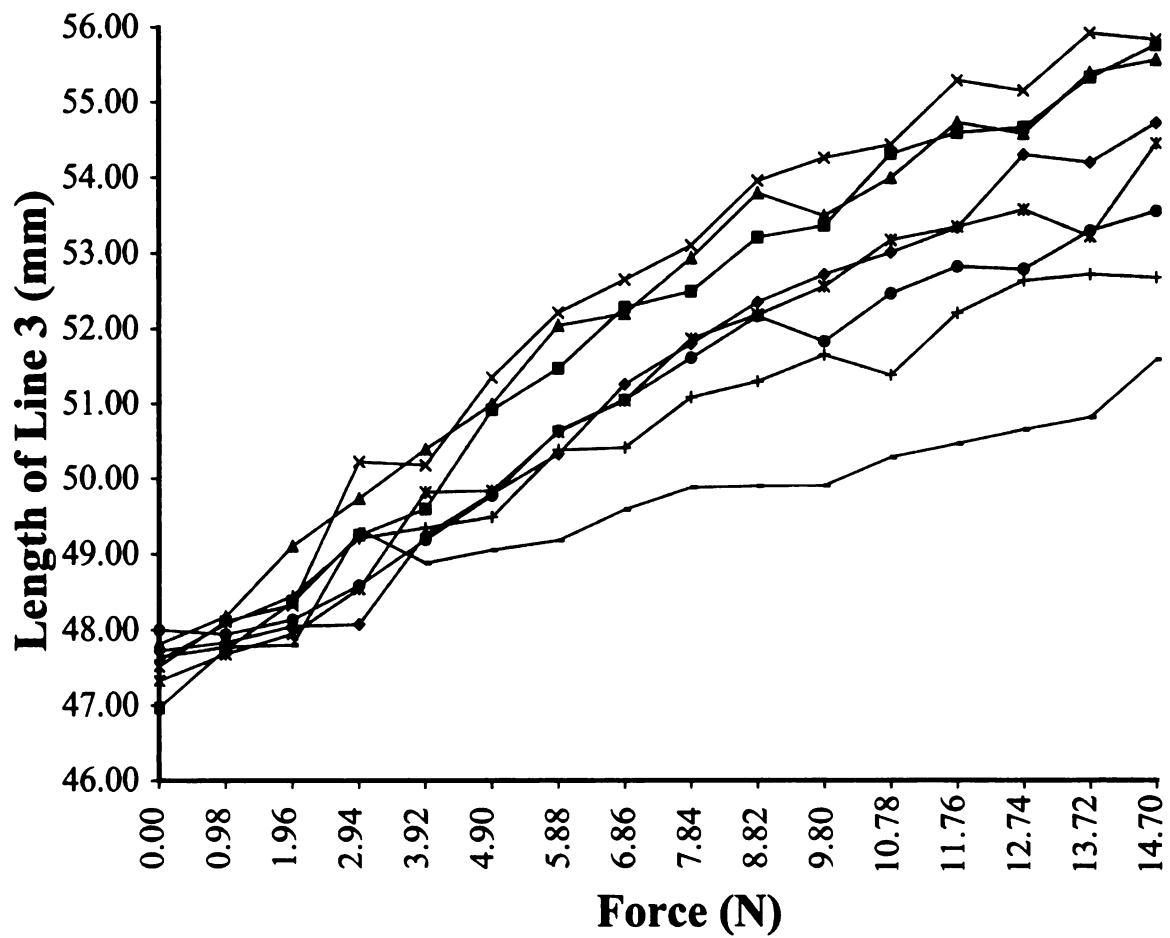


Figure 3c: Effect of force (Newtons) and angle (degrees) on the length of Line 3.
 ◆ 0 degrees; ■ 10 degrees; ▲ 20 degrees; × 30 degrees; * 40 degrees;
 ● 50 degrees; + 60 degrees; and – 70 degrees.

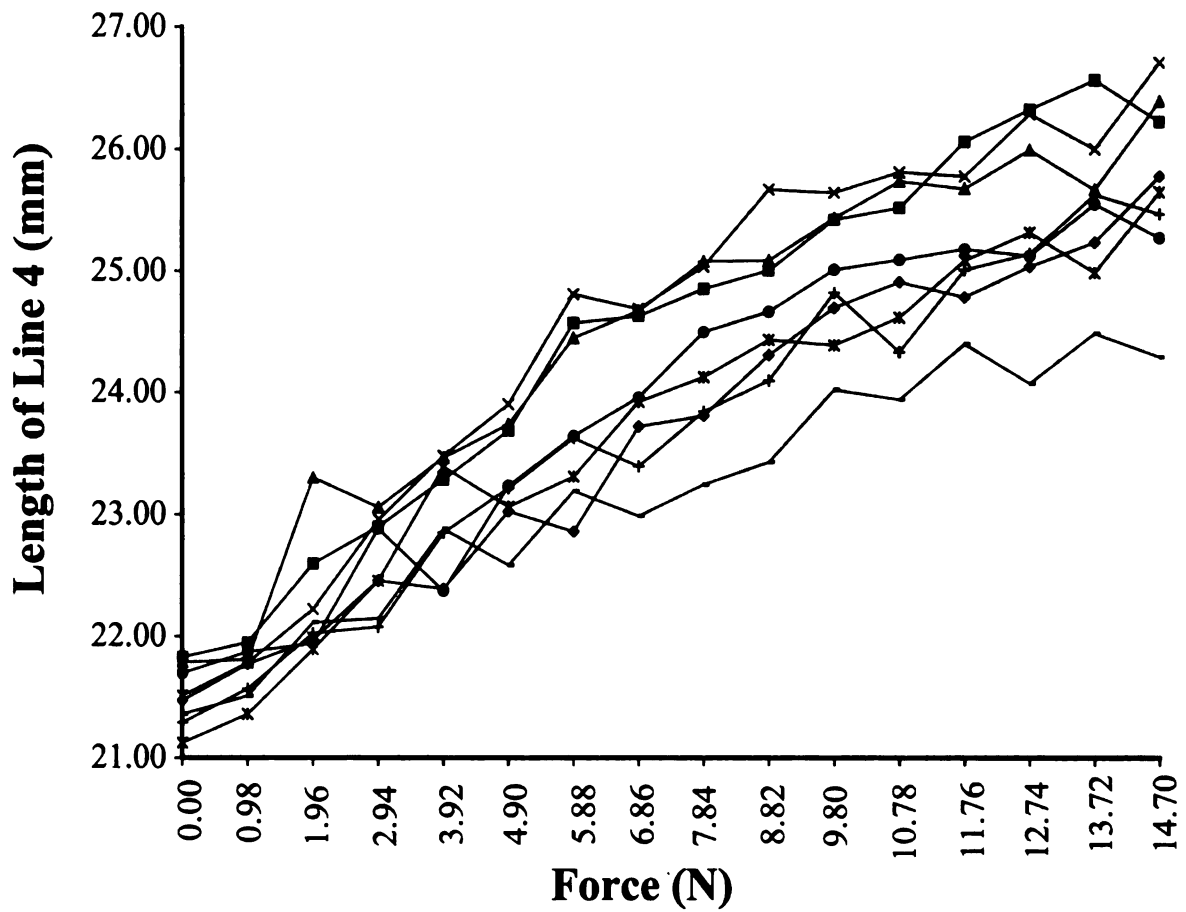


Figure 3d: Effect of force (Newtons) and angle (degrees) on the length of Line 4.
 ◆ 0 degrees; ■ 10 degrees; ▲ 20 degrees; × 30 degrees; * 40 degrees;
 ● 50 degrees; + 60 degrees; and – 70 degrees.

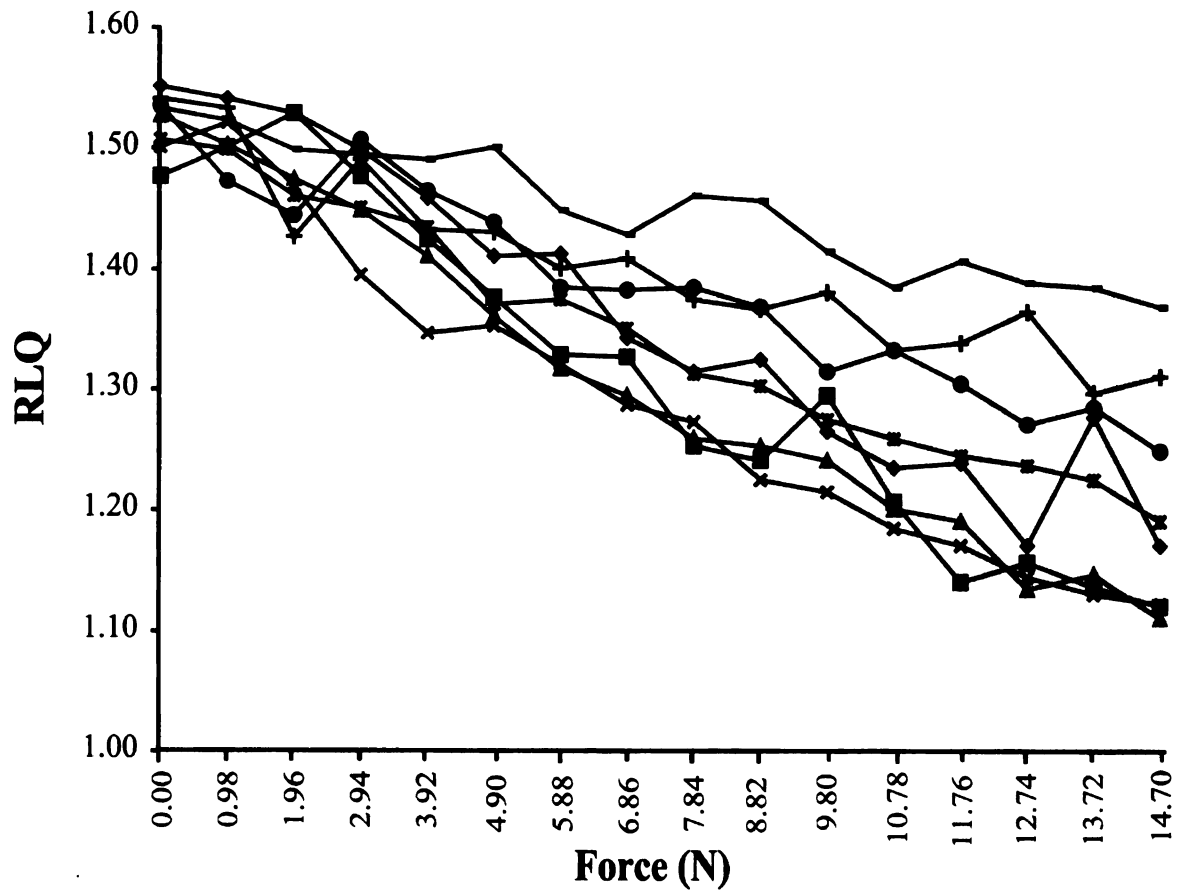


Figure 4: Effect of force (Newtons) and angle (degrees) on Right to Left Quotient (RLQ). ◆ 0 degrees; ■ 10 degrees; ▲ 20 degrees; × 30 degrees; * 40 degrees; ● 50 degrees; + 60 degrees; and – 70 degrees.

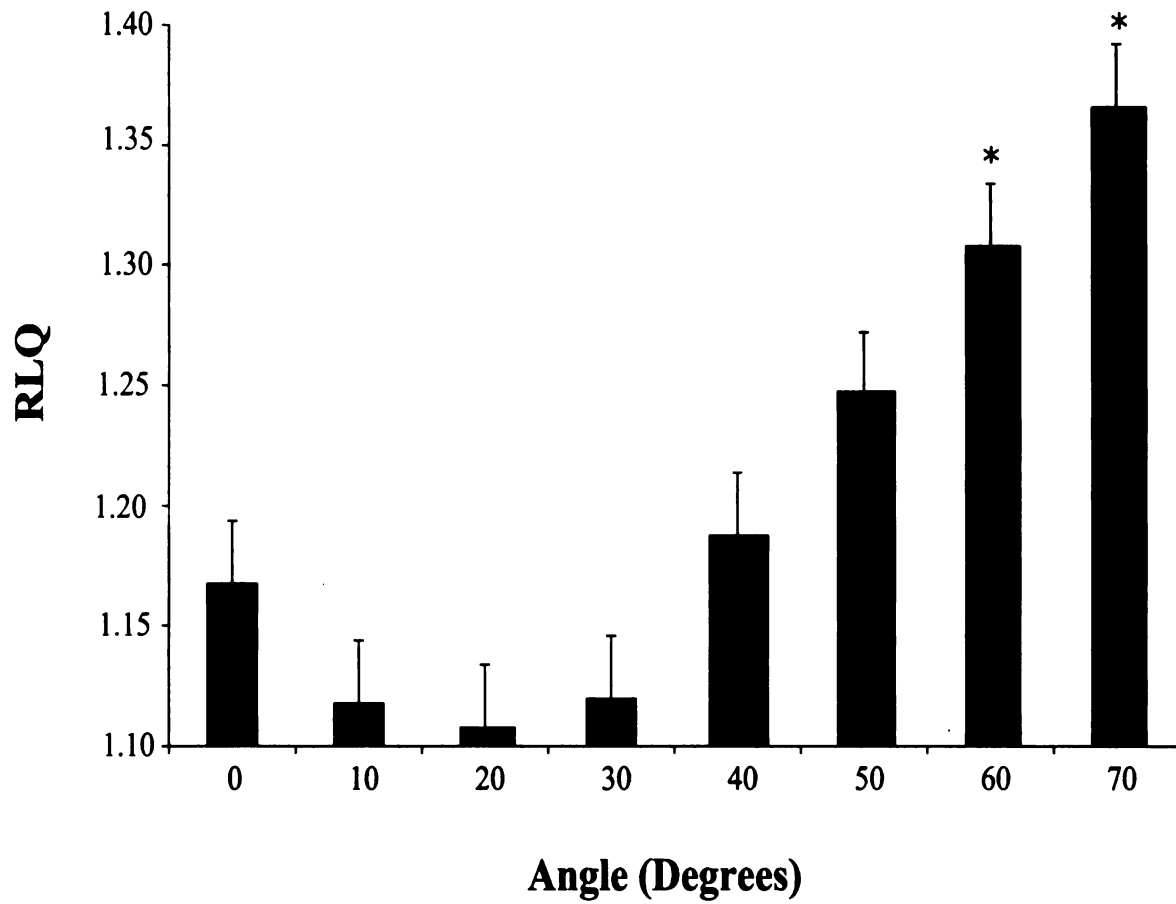


Figure 5: Effect of angle (degrees) on to Left Quotient (RLQ) at a force of 14.7 Newtons. * Significant difference from 10, 20, and 30 degrees.

abduction, and suggest that proper placement of NMPs in the CAD muscle may result in earlier and greater arytenoid abduction.

The CAD muscle has a medial and lateral neuromuscular compartment (NMC) (Figure 2) (Cheetham *et al.* 2008). In our study, angles of 0-30 degrees correspond to the lateral NMC whilst angles 40-70 degrees correspond to the medial compartment. In dorsal (line 4), lateral (line1), and dorsolateral (lines 2 and 3) directions, force applied at angles 0-30 degrees produced a greater degree of abduction than angles 40-70 degrees. This was confirmed by the RLQ. At 0 N of force applied there was no significant difference in abduction as measured by any of the lines and RLQ, demonstrating that in the experiment, previous abduction of the left arytenoid did not affect subsequent abductions. At 7.84 N for lines 2, 3, and 4, and RLQ, and at a force of 11.76 N for line 1, the differences in abduction of the left arytenoids became significant. Greatest abduction of the arytenoid required 14.70 N of force applied at an angle of 30 degrees. This same force applied at 40-70 degrees resulted in submaximal abduction. These data imply that placement of a nerve-muscle pedicle graft in the lateral NMC would be more effective in terms of arytenoid abduction than placing the grafts in the medial NMC. This confirms previous findings in a neuroanatomical study of the CAD muscle (Cheetham *et al.* 2008). Because nerve sprouting does not cross fascial planes (Tucker 1979), and the equine CAD has two distinct NMCs separated by connective tissue (Cheetham *et al.* 2008), nerve sprouts from a NMP placed in the medial compartment will likely not cross over and reinnervate the lateral compartment.

We are not suggesting that placement of a NMP in the medial compartment is of no value. Indeed, it is likely that reinnervation of both NMCs is required for optimal CAD function. During abduction most of the movement occurs in a lateral and dorsal direction; however, the arytenoid cartilage can also move in a caudal direction (Cheetham *et al.* 2008). We do not have information about arytenoid movement in a caudal direction, therefore it is possible that the medial and lateral NMCs have differing actions on the movement of the arytenoid in a caudal direction. Further studies are required to evaluate the effect of force-angle interactions on three-dimensional movement of the left arytenoid.

In the experience of our surgeons, in the majority of horses, four nerve-muscle pedicles are available for grafting and there is sufficient space for two nerve-muscle pedicle grafts in the lateral NMC. To place more than 2 of these grafts, or use pedicles that are too large, in the lateral NMC could result in fibrosis and excessive scar tissue, negatively affecting successful reinnervation and the muscle's ability to abduct the left arytenoid cartilage (Tucker 2006). Therefore it would be more beneficial to place the remaining nerve-muscle pedicles in the lateral portion of the medial NMC. It is fortunate that the approach to the lateral NMC and the lateral portion of the medial NMC of the CAD muscle is easier than the approach to the more medial aspects of the CAD muscle. We have found anatomic variability in the size of the medial and lateral NMCs between horses. This means that surgeons must adapt to these variations in each case, bearing in mind the importance of the lateral NMC.

It has been suggested that in the case of neurotization, reinnervation of muscle by transected nerves, the reinnervation occurs most easily at the site of the native motor endplates (Goding Jr. 2005). In the equine CAD muscle, the 2 NMCs each have a single motor end plate band arranged along the caudal aspect of the lateral compartment and the medial aspect of the medial compartment (Cheetham *et al.* 2008). Thus, placement of the NMP in the middle of the lateral compartment and on the lateral aspect of the medial compartment as recommended above, creates a distance between some of the nerve pedicles and the native motor and plates. When a NMP is placed in a denervated muscle, most reinnervation occurs by axon sprouting from motor end plates located in the donor pedicle (Gray *et al.* 1997). A muscle pedicle of 2-3mm² contains approximately 60% of the motor end plates for that nerve (Tucker 2006). Reinnervation of motor endplates in the recipient muscle by nerve endings transected at the edge of the muscle pedicle is also possible (Gray *et al.* 1997). In both cases, the nerve "sprouts" must either reinnervate native motor end plates or generate new (ectopic) motor end plates. When a transected nerve is implanted near native motor end plates, most new axons innervate these native motor end plates, while nerve implantation distant to native motor end plates results in significantly more ectopic motor end plates (Payne and Brushart 1997). The total number of motor end plates is independent of implant location (Payne and Brushart 1997). Taken together, these data suggest that the location of NMPs relative to native motor endplates is unimportant.

Results of the current study also have relevance to the placement of the prosthetic laryngoplasty suture in the treatment of RLN. Cranially, the suture penetrates the muscular process of the left arytenoid cartilage, and caudally the suture is placed around the posterior aspect of the cricoid cartilage and into a position 2-3 cm cranial to its caudal border and 1 cm lateral to the spine of the cricoid cartilage (Stick 2006). Placed in this manner, the force generated by the suture is at angles between 20 and 40 degrees. All of our measurements indicate that this direction of pull resulted in maximum arytenoid abduction, probably contributing to the success of this procedure. While a more lateral placement of the suture through the posterior edge of the cricoid cartilage would also result in maximum abduction, this is not advised because of anatomical considerations. The curvature of the caudal border of the cricoid cartilage allows slipping of the suture along the posterior aspect of the cartilage and subsequent loosening of the entire prosthesis. In the clinical setting, lateral placement has been associated with prosthetic failure.

To summarize, this study demonstrates that forces applied to the muscular process of the arytenoid cartilage in a direction corresponding to the lateral NMC of the CAD results in greater arytenoid abduction than when these same forces are applied in a more medial direction. This suggests that NMP grafts should be placed in a manner as to confer most success in reinnervating the lateral NMC. These data should be helpful to surgeons when performing NMP grafts and should result in a more successful procedure.

6. Effect of electrostimulation on upper airway function in laryngeal hemiplegia affected horses treated with a nerve muscle pedicle graft.

a. Introduction and Aims

Laryngeal reinnervation is used to treat people with laryngeal denervation (Kingham *et al.* 2006; Su *et al.* 2007; Tucker 1989; Tucker and Rusnov 1981). In horses, laryngeal reinnervation using a first cervical nerve-omohyoideus muscle pedicle graft technique can be as effective in the restoration of racing performance as prosthetic laryngoplasty and offers a more physiologic treatment with fewer complications (Fulton *et al.* 1991; Fulton *et al.* 2003). However, the technique has not gained wide acceptance due to the extended recovery time (Fulton *et al.* 2003). The overall goal of this research was to determine if, in horses with LH, postoperative electrostimulation of the reinnervated muscle allows earlier return to athletic performance without risk of coughing, aspiration, and respiratory infection.

Electrostimulation of skeletal muscle after denervation has been studied extensively in humans and laboratory animals (Gondin *et al.* 2006; Maffiuletti *et al.* 2006; Marqueste *et al.* 2004; Poortmans and Wyndaele 2002; Vitenzon *et al.* 2005). Within 5 weeks, denervated muscle has a dramatic loss of size and ability to

contract and carry out its function. For example, within 5 weeks, denervated extensor digitorum longus muscles of rats lose 66% of mass, 91% of force, and 76% of fiber cross-sectional area (Dow *et al.* 2004). Electrostimulation of these muscles can completely prevent these impairments (Dow *et al.* 2004). Atrophy of denervated muscle impairs its ability to become reinnervated (Cole and Gardiner 1984; Fu and Gordon 1995); therefore the process of electrostimulation may aid the reinnervation process. This possibility has important implications in equine LH, where the denervated CAD muscle is responsible for the loss of upper airway function.

The electrostimulation protocol, muscle fiber type, and species differences have important effects on muscle responses to stimulation (Dow *et al.* 2004). Generally, frequent submaximal stimulation encourages slow fiber type growth, while less frequent maximal contractions encourage fast twitch fibers, muscle fiber hypertrophy, and faster force recovery. For example, in the denervated extensor digitorum longus muscle of rats, a typical fast twitch muscle, near normal force was maintained with 100 contractions per day for 5 weeks, while 800 contractions per day caused muscle damage (Dow *et al.* 2004). In another study involving denervated (for 6 to 24 weeks) human quadriceps muscle, a mixed fiber type muscle, 80 contractions per week for 8 weeks were sufficient to generate a 20% increase in muscle mass (Dudley *et al.* 1999). The equine CAD muscle is a mixed fast (55%) and slow twitch (45%) skeletal muscle (Hoh 2005), similar to the human quadriceps muscle (Green *et al.* 1999). In this study we have chosen an

electrostimulation protocol that would be expected to increase muscle mass, force, and fiber cross-sectional area in denervated fast and mixed fiber-type muscles in rats and humans respectively (Dow *et al.* 2004; Dudley *et al.* 1999).

Hypothesis

The central hypothesis of this study is that post-operative electrostimulation of the nerve muscle pedicle graft and recipient cricoarytenoideus dorsalis muscle results in greater and earlier decrease in upper airway obstruction in exercising horses with laryngeal hemiplegia in comparison to unstimulated controls.

Specific Aims

1. To measure trans-upper airway pressure in exercising horses before and after induction of LH
2. To measure trans-upper airway pressure in exercising horses with LH before and 30 days after placement of neuromuscular pedicle grafts
3. To determine if, relative to controls, postoperative electrostimulation of the nerve muscle pedicle grafts and recipient cricoarytenoideus dorsalis muscle decreases trans-upper airway pressure and improves laryngeal function at 44, 58, 72, 86, 100, 114, 128, 142, 156 and 170 days after nerve muscle pedicle graft surgery.

b. Materials and Methods

Horses

Four adult horses were used in this study. All horses were administered an anthelmintic and vaccinated against eastern and western equine encephalitis, tetanus, equine influenza, and rhinopneumonitis before the start of the study. Endoscopic evaluation of the upper airway, including the trachea, was performed when horses were at rest and during high-speed exercise. To be included in the study, all horses had endoscopically normal upper airways. This study was approved by the Institutional Animal Care and Use Committee at Michigan State University.

Experimental Protocol

The four horses were randomly divided into two groups of two horses: an electrostimulation group (principals) and a control group. Upper airway endoscopy was performed at rest, and before and after surgical procedures to document the induction of LH or postoperative complications. Horses in the electrostimulation group received stimulation of the nerve muscle pedicle graft as described below. The control group did not receive stimulation. Between measurement periods, all horses were maintained on pasture. I planned to make measurements at 13 time periods: before (baseline) and 30 days after left recurrent laryngeal neurectomy; and at 2 week intervals (30, 44, 58, 72, 86, 100, 114, 128, 142, 156 and 170 days) after nerve muscle pedicle graft surgery. Principal horses

were supposed to receive electrostimulation for 6 months, starting at 30 days after nerve muscle pedicle graft surgery, however this could not be achieved in any of the horses for technical reasons, which will be discussed later. The timeline of measurements and electrical stimulation actually used is shown in figure 6. During each measurement period, datum were collected from horses exercising at their individual maximum heart rate determined as previously described (Tetens *et al.* 1996). Following a 4-minute warm-up period, horses were exercised at a treadmill speed corresponding to their individual maximum heart rate for 2 minutes or until the horse could no longer maintain its position on the treadmill. Trans-upper airway pressure measurement was performed and endoscopy was recorded on 2 separate days. After the last measurement was taken, horses were euthanized. Each of the larynges and implanted first cervical nerves were subjected to a postmortem evaluation to assess the degree of denervation, degeneration and reinnervation.

<u>START</u>	<u>Day</u>	<u>Tests and/or Procedures Performed</u>
	0	<u>Baseline</u> Left recurrent laryngeal neurectomy Endoscopy (standing and on treadmill) Inspiratory Pressure
	30	Nerve muscle pedicle graft (NMP graft) Endoscopy (standing and on treadmill) Inspiratory Pressure
	60	<u>30d post NMP graft</u> Start of electrostimulation protocol Endoscopy (standing and on treadmill) Inspiratory Pressure
	74	Endoscopy (standing and on treadmill) Inspiratory Pressure
	88	Endoscopy (standing and on treadmill) Inspiratory Pressure
	102	Endoscopy (standing and on treadmill) Inspiratory Pressure
	<u>Euthanized horses 1 & 2</u>	
	116	Endoscopy (standing and on treadmill) Inspiratory Pressure
	130	Endoscopy (standing and on treadmill) Inspiratory Pressure
	144	Endoscopy (standing and on treadmill) Inspiratory Pressure
	158	Endoscopy (standing and on treadmill) Inspiratory Pressure
<u>END</u>	<u>Euthanized horses 3 & 4</u>	

Figure 6: Timeline in days from day 0 through to the end of the electrostimulation research project.

Measurement of Trans-Upper Airway Pressure

A fenestrated polyethelene catheter, placed via the right nostril into the mid-cervical region, was used to measure tracheal pressure. Inspiratory and expiratory trans upper airway pressure was measured as the pressure difference between tracheal and atmospheric pressure (Robinson *et al.* 2006).

Surgical Procedures

For all surgical procedures, all horses were administered peri-operative antibiotics (potassium penicillin 22,000 IU/kg i.v. q.i.d. and gentamicin sulphate 6.6 mg/kg i.v. s.i.d.) and an anti-inflammatory agent (flunixin meglumine 1.1 mg/kg i.v. b.i.d.).

Left Recurrent Laryngeal Neurectomy

Each horse was induced into general anesthesia. The left, mid-cervical region was clipped and prepared for aseptic surgery. An incision was made immediately ventral to the jugular vein and the left recurrent laryngeal nerve was identified and isolated. Identification of the correct nerve was confirmed by endoscopic visualization of abduction of the left corniculate process during nerve stimulation by use of a hemostatic forceps. After isolation, a 2.5 cm segment of the nerve was transected and removed (Derksen *et al.* 2001).

Nerve Muscle Pedicle Graft Procedure

The nerve muscle pedicle graft was inserted by use of a technique described by Fulton *et al.* (1991). A 12-cm linear skin incision was made along the ventral border of the linguofacial vein. Blunt dissection between the omohyoideus muscle and linguofacial vein was used to identify the first cervical nerve as it travels over the left cricopharyngeus muscle to its insertion in the omohyoideus muscle. Cranial retraction of the cricopharyngeus muscle allows visualization of the cricoarytenoideus dorsalis muscle in which three 1-cm openings were created between and parallel to the muscle fibers in the lateral compartment of the CAD muscle. Three nerve muscle pedicle grafts were created at the point of entry of a branch of the first cervical nerve into the omohyoideus muscle. Pedicles of muscle approximately 5 x 5 mm were removed with the nerve intact and transposed to the previously created openings in the cricoarytenoideus dorsalis muscle. Two simple interrupted sutures of 4-0 polydioxanone were used to secure the cranial and caudal aspect of the pedicle. A length of insulated wire (Teflon-coated stainless steel , 36 AWG) was wrapped around the nerve and the two ends clamped close to the nerve to ensure the wires stayed in place. The wire was tunneled underneath the skin and exited in the midcervical region. This region was bandaged for 96 hours post-operatively and changed when indicated. The omohyoideus muscle and lingual facial vein was apposed with 2-0 polydioxanone suture, and the skin was closed in a routine manner.

Electrostimulation Protocol

Thirty days after placement of the nerve muscle pedicle grafts, the left cricoid-arytenoideus dorsalis (CAD) muscle was stimulated every other day for a target period of 60 days as follows: The ends of the electrodes were attached to a N EMS +2 neuromuscular stimulator (Rehabicare, New Brighton, MI). An endoscope was placed to view the larynx. A 50 Hz, 0.4 milli-seconds duration, 0.5 V biphasic stimulus was applied for 5 seconds. Eight sets of 12 stimuli were administered (total of 96 stimuli) using a 5 second/5second contraction /rest ratio with 2 minutes of rest between sets. This stimulation protocol took 30 minutes to complete, which is brief enough to be practical in a future field setting.

Post-mortem Evaluation

The left and right CAD muscles were dissected out, measured, weighed and their volume was calculated. The CAD muscle pairs were then packed in ice and sent away for neuromuscular evaluation. When evaluating the tissue samples for this study several approaches were taken according to the criteria of interest. The presence of angular atrophy, anguloid atrophy, central nuclei, acute necrosis, macrophages, myotubes, pyknotic nuclei, vasculitis, fibrosis, and fiber size variation were determined by evaluating H&E stained tissues. Each slide was analyzed in 12 consecutive sections at 20 X power of 3 rows and 4 columns to ensure completeness of data collection. The individual criteria was ranked from 0 to 3, 0 indicating no change, 1 indicating the presence of change, 2 indicating mild to moderate change and 3 indicating severe change. Each of the 12 sections was

then averaged to give a final value for each of the criteria. A number of other criteria were evaluated on the same number scale but as a whole slide rather than in 12 sections. These included the presence of weird glycogen and nerve demyelination using the periodic acid-Schiff (PAS) stain, the degree of fiber-type grouping using the ATPase stain, the presence of moth-eaten fibers and targets using the NADH stain, the presence of intracellular and extracellular lipids using the Oil Red O (ORO) stain, the presence of red stippling using the Acidphosphatase stain, and the presence of end plates using the esterase stain. Minimum fiber diameter was evaluated by viewing three sections on each slide. Sections were determined by the general appearance of one large, the next largest and one of the smallest group of fibers and measuring the minimum diameter all fibers present on each of these individual views at 20X. Then an average was taken of these three views to give a final score. Finally a score was determined for denervation by assessing angular atrophy, anguloid atrophy, pyknotic nuclei nerve bundle de-myelination. Degeneration was evaluated by assessing acute necrosis, macrophages, fibrosis red stippling (acidphosphatase). Reinnervation was evaluated by assessing central nuclei, myotubes, fiber-type grouping, moth-eaten fibers, target fibers and end plates. By adding the averages for the categories and dividing them by the number of categories a score could be attributed to each category for the purposes of comparison.

c. Results

Endoscopic evaluation at rest and on the treadmill confirmed that all horses became grade IV left laryngeal hemiplegia affected horses following left recurrent laryngeal neurectomy.

i. Inspiratory Pressure

In all horses there was an increase in inspiratory pressure following induced left sided laryngeal hemiplegia. The results of this can be seen in figure 7.

ii. Endoscopic Observations

None of the horses evaluated experienced a return to normal function and only one horse (horse 3) had any evidence of reinnervation. This was observed 128 days after the nerve muscle pedicle graft procedure had been performed. When lifting the head during endoscopic examination of the larynx there was a small flicker from the left arytenoid cartilage. Furthermore, there was slight abduction during inhalation when the exercised at maximum heart rate on the treadmill. During exercise the arytenoid abducted initially but then returned to the position as it was 30 days post NMP graft.

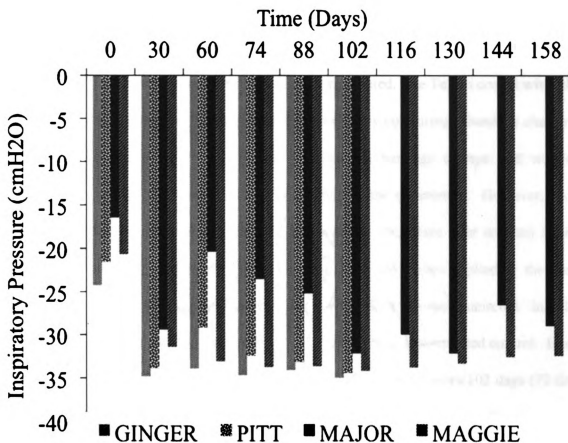


Figure 7: Mean inspiratory pressure measurements (cmH₂O) for the 4 horses recorded at maximum heart rate on the treadmill. 0 days is baseline prior to left laryngeal neurectomy (at 0 days), then 30 days after left laryngeal neurectomy (at 30 days), then 30 days following NMPPG (at 60 days) and then every 14 days thereafter.

iii. Complications:

There were a number of unforeseen technical problems that prevented me from completing the experiment as planned. In both of the stimulated horses, the wires were pulled out or transected. The Teflon coated wires that exited the skin in horse 1 were inadvertently cut during a bandage change in the hospital. This was an unscheduled bandage change and was not witnessed by any of the team conducting the experiment. However, given the extensive damage to the first cervical nerve (see later section) I think that a significant amount of traction may have been applied to the wires resulting in tightening and damage to the nerve that they encircled. Initially, this was not recognized and horse 1 became a non-stimulated control. Horse 2 became the stimulated principal. Horse 2 lost the wires 102 days (72 days after nerve muscle pedicle graft) into the experiment. There was no explanation for this, but it was later discovered that horse 3 had developed a penchant for rubbing and nibbling on the exposed wires of her pasture mates.

Horses 3 and 4 lasted for longer in the study, it was not until day 158 that horse 4 lost the wires (128 days after nerve muscle pedicle graft), an eyewitness account clearly identified horse 3 as the saboteur! At this point in time both horses were euthanized and sent for post-mortem examination and sample collection.

iv. *Post Mortem Findings*

At post-mortem the left and right CAD muscles were dissected out and visually inspected, all of the NMP grafts were in place and covered with a fibrous tissue layer. The first cervical nerve was dissected free and histologically evaluated. Results of the histological evaluation of the first cervical nerve were as follows, as reported by the pathologist:

Horse 1:

“There was marked collagen deposition within the perineurium and epineurium, and subdivision of nerve fascicles. Most of the fascicles were disrupted by collagen, with extensive loss of nerve fibers; many of the individual nerve fibers are no longer within an apparent fascicle. There is essentially no myelin surrounding the nerve fibers, and there are abundant ‘Bungner’s bands’ present in the remnant fascicles. Within the most intact fascicles there are scattered markedly enlarged nerve fibers (spheroids).”

Horse 2:

“There are multiple nerve fascicles present, with a normal number and density of myelinated fibers present.”

Horse 3:

“The epineurium is moderately expanded by mature collagen. Multiple nerve fascicles are evident, and the majority of these fascicles contain a normal number and density of myelinated nerve fibers within each fascicle. Other fascicles have a moderate loss of myelinated fibers, with scattered foci of proliferating Schwann cells forming ‘Bungner’s bands’, and infiltration with small numbers of macrophages. There are two foci of inflammation, comprised of moderate numbers of eosinophils and lymphocytes, with fewer epithelioid macrophages.”

Horse 4:

“The epineurium is moderately expanded by mature collagen. Multiple nerve fascicles are evident, and the majority of these fascicles contain a normal number and density of myelinated nerve fibers within each fascicle. Adjacent to this the nerve bundle, there are several fascicles that are markedly atrophied, and enmeshed within abundant collagen. The remaining nerve fibers within the remnant fascicles are devoid of myelin. Along the edge of the sample, there is a thin rim of epithelioid macrophages, admixed with small numbers of lymphocytes and occasional eosinophils.”

The CAD muscles were weighed and their volume calculated by measuring the volume of water displaced in a 250 ml flask. The results are shown in Table 1, horse 1 demonstrated a loss of half of the weight of the muscle and

a loss of half of the volume of the muscle compared with the right side (control), both horses 3 and 4 demonstrated a mild decrease in both weight and volume of the CAD muscle when compared with the right CAD muscle (control). Horse 2 had no differences between the left and right CAD muscle.

The CAD muscle pairs were sent for neuromuscular tissue evaluation and the results are summarized in Table 2. All horses showed signs of denervation, degeneration and reinnervation when compared with the right CAD muscle (control). Horse 1 experienced the largest amount of denervation and horse 3 showed the least. Horse 1 and 4 suffered from the largest amounts of muscle degeneration and horse 3 experienced the least amount. When the degree of reinnervation was evaluated, horse 3 showed the largest amount, followed by horse 1 with horse 4 experiencing the least amount of reinnervation. Please see figure 8 for examples of these histological findings in the muscle and the nerve.

d. Discussion and Conclusions

Technical complications involving the wires and the first cervical nerve including premature severing of the wires and inappropriate placement around the nerve

	Horse 1		Horse 2		Horse 3		Horse 4	
CAD	<i>Wt (g)</i>	<i>Vol (cc)</i>	<i>Wt (g)</i>	<i>Vol (cc)</i>	<i>Wt (g)</i>	<i>Vol (cc)</i>	<i>Wt (g)</i>	<i>Vol (cc)</i>
Left	6.13	3.50	7.92	5.50	8.45	8.00	8.55	9.00
Right	8.60	6.50	7.79	5.50	9.45	9.50	11.15	12.00

Wt: Weight; (g): grams; Vol: Volume; (cc): centiliters; CAD: Cricoarytenoideus dorsalis muscle

Table 1: Weight and volume of the left and right cricoarytenoideus dorsalis muscle of the 4 horses

Muscle Region	Horse 1			Horse 2			Horse 3			Horse 4		
	DN	DG	RN	DN	DG	RN	DN	DG	RN	DN	DG	RN
L Cra Lat	1.42	0.85	1.00	0.96	1.25	0.49	0.83	0.50	0.67	1.40	1.37	0.35
L Cau Med	1.36	1.42	0.76	1.29	0.73	0.85	0.65	0.50	1.37	1.02	0.94	0.26
<i>Combined for L CAD</i>	2.78	2.27	1.76	2.25	1.98	1.34	1.48	1.00	1.14	2.42	2.31	0.61
R Cra Lat	0.02	0.25	0.50	0.00	0.27	0.17	0.27	0.05	0.35	0.00	0.27	0.51
R Cau Lat	0.02	0.29	0.83	0.00	0.50	0.17	0.04	0.33	0.17	1.14	0.27	0.40
<i>Combined for R CAD</i>	0.04	0.54	1.33	0.00	0.77	0.34	0.31	0.38	0.52	1.14	0.54	0.91
Arytenoid Stimulation	None			None			Yes at the end of the experiment			None but no collapse seen		
Endoscopy	No change			No change			None			No change		
UA IP	No change			No change			Some abduction at exercise			No change		

Where L is left; R is right; Cra is cranial; Cau is caudal. CAD is cricarytenoideus dorsalis muscle; UA IP is upper airway inspiratory pressure; DN is denervation score; DG is degeneration score; RN is reinnervation score

Table 2: Mean neuromuscular histology score for each muscle region analyzed.

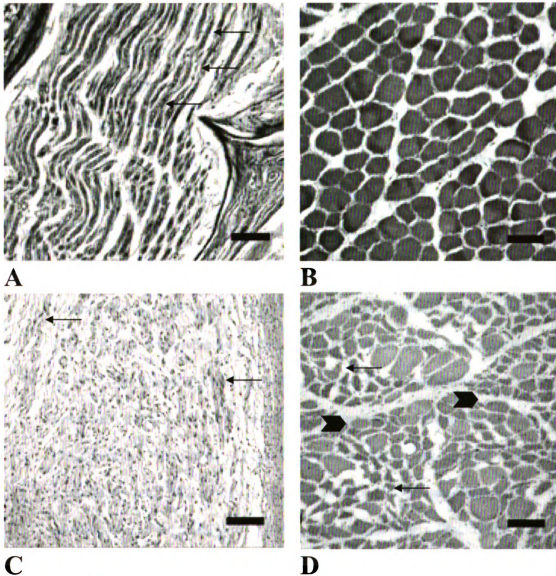


Figure 8: A) Longitudinal histologic section of normal peripheral nerve, Horse No. 3 Luxol Fast Blue (LFB) histochemistry. The nerve is comprised of numerous individual myelinated nerve fibers. The blue represents the myelin sheaths surrounding the nerve fibers (arrow). Bar = 100 μ m. B) Histologic cross-section of normal skeletal muscle (left CAD), horse No. 3 Hematoxylin and eosin histochemistry. The density, size, and shape of the individual myofibers is normal within the muscle. Note the normal peripheral localization of the myofiber nuclei. Bar = 100 μ m. C) Longitudinal histologic section of abnormal peripheral nerve histology, Horse No. 1 Luxol Fast Blue (LFB) histochemistry. There are no normal nerve fibers present, and no histochemically detectable myelin in the nerve. The tissue is comprised of numerous linear bundles of proliferating Schwann cells ('Bungner's bands, arrow). Bar = 100 μ m. D) Histologic cross-section of abnormal skeletal muscle (left CAD). Hematoxylin and eosin histochemistry. There are numerous markedly angular, and atrophic muscle cells (arrow) interspersed with scattered hypertrophic myocytes with internal nuclei (arrowhead). Bar = 100 μ m.

doomed the experiment. Loss of the wires in all horses led to early euthanasia of the horses. In horses 1 and 2 this resulted in there being insufficient time for the NMP graft to reinnervate the CAD and it was not long enough for any reasonable evaluation of the efficacy of the electrostimulation protocol. In horses 3 and 4, loss of wires occurred later. This left enough time for the NMP graft to reinnervate the CAD muscle in horse 3 (128 days).

Placement of the wires around the first cervical nerve also resulted in significant complications. To secure the wire we used a small stainless steel clamp (Securos⁵⁵) to secure the loop. In horse 1 histologic evaluation of the nerve at the level of the clamp showed that the nerve was severely damaged. In horse 2 however, there were few histologic abnormalities indicating that the wire placement can be effective. These 2 horses are interesting because horse 1 had a completely damaged nerve and consequently a CAD muscle with no neural stimulation. In contrast, horse 2 had a CAD muscle with a functioning NMP graft, which had been in place for 74 days. When these 2 pairs of CAD muscle were examined, there was a significant difference. Horse 1 had a much smaller (volume and weight) atrophied left CAD muscle compared to the right (control) CAD, whereas horse 2 had similar volume and weight of the right and left muscles (Table 2 and Figure X). The results of the histological neuromuscular evaluation generally support the gross observations. Horse 2 had mild evidence of degeneration and denervation but very little signs of reinnervation (Table 1). This

⁵ Securos 443 Main Street, PO Box 950, Fiskdale, MA 01518

is what would be expected given the time frame; 12 weeks is the earliest time that reinnervation has been seen (Fulton *et al.*, 2003) and the grafts had only been in place for 74 days (10 weeks) so it is unlikely that any reinnervation would have been seen. Apparently, effective placement of the NMP graft was sufficient to prevent significant degeneration and denervation in the left CAD muscle of horse 2. However, horse 1 that had no neurologic supply to the CAD muscle had lost approximately half of the weight and volume of the left CAD muscle when compared to the right side, which is what would be expected for a muscle that has not had any innervation for 102 days. For example, within 5 weeks, denervated extensor digitorum longus muscles of rats lose 66% of mass, 91% of force, and 76% of fiber cross-sectional area (Dow *et al.* 2004). Furthermore, this horse had more evidence of denervation and degeneration than any of the other horses.

In horses 3 and 4, the left CAD weighed less than the right and possessed a smaller volume than its control (Table 2), horse 3 demonstrated a smaller discrepancy than horse 4. This difference between the two was reflected by the fact that there was more damage to the nerve in horse 4 than horse 3. On histologic neuromuscular evaluation, horse 3 showed small amounts of degeneration and denervation of the CAD muscle with the highest amount of reinnervation occurring. In contrast, horse 4 showed the least amount of reinnervation, the most degeneration and a substantial amount of denervation. These data would suggest that horse 3 was beginning to benefit from a healthy NMP graft and the associated reinnervation whereas the more significant damage to the first cervical nerve in horse 4 was

sufficient to prevent any reinnervation. It is apparent that horse 3 was beginning to see a return to function and acceptance of the NMP graft.

At no point did electrostimulation of horses 2 and 4 result in abduction of the left arytenoid as reported in Fulton's pilot study (Fulton *et al.*, 2003). Instead, we observed other muscles such as the cricopharyngeus, cricothyroideus and unidentified muscles of neck contracting when the electrostimulation was applied. This would indicate that the current was not applied specifically to the first cervical nerve and was instead also transmitted to the surrounding musculature. There is a remote possibility that the lack of abduction could have resulted from synkinesis (Tucker, 2005) but this seems unlikely due to the short time frame.

As expected, inspiratory pressure increased in all horse demonstrating that left recurrent laryngeal neurectomy caused upper airway obstruction. Inspiratory pressure did not change following NMP graft in any of the horses. In horses 1 and 4 this was probably due to first cervical nerve damage due to electrode placement. However, in horses 2 and 3 this is probably because there was insufficient time between NMP graft placement and measurements. Furthermore, videoendoscopy indicated that there was no active left arytenoid movement in all horses except horse 3. In this horse there was a small 'flicker' with head elevation and an active arytenoid abduction during exercise. This correlates well with the histologic evidence of an undamaged first cervical nerve and histologic evidence of reinnervation. It should be noted that horse 4 demonstrated some persistent

resistance to dynamic collapse of the left arytenoid, which could have resulted from excessive fibrous tissue at the surgery site. A finding that could be used to explain the lack of success associated with the procedure in this horse (Tucker, 1989). Horse 3 began to show some promise at the end of the experiment, response to head lift and also resistance to inspiratory collapse of the left arytenoid at the initial period of exercise, which soon became overcome by the negative inspiratory pressure. This supports the theory that horse 3 had a successful NMP graft that would have gone on to do well given enough time.

In conclusion, unfortunately, it was not possible for us to test our hypothesis. We cannot draw conclusions on whether electrostimulation speeds up the time taken for a NMP graft to reduce upper airway obstruction associated with laryngeal hemiplegia.

7. Lessons Learnt: A Look to the Future

In two out of the four horses placement of the electrodes in the manner that we used resulted in significant damage to the first cervical nerve rendering it dysfunctional. Furthermore, the electrodes that we used were not sufficiently well insulated, making it impossible to direct electrostimulation accurately. We did not tie the Teflon wires as Fulton reports instead we used a small metal clamp to hold the two ends together as they wrapped around the nerve. I believe that the metal clamp provided two problems; firstly, it was not insulated so it allowed a current to flow to whatever structure it lay in contact with. Secondly, it was relatively heavy and would put traction on the nerve, which may have caused damage, alternatively, any swelling of the nerve or slipping of the wire as the head and neck moved may have lead to significant tightening around the nerve and subsequent damage. The size of the clamp may have been responsible the significant amount of fibrous tissue that was found at post-mortem, which is disrupting to the process of reinnervation (Tucker, 1989). Perhaps tying the nerves would not have resulted in the damage to the nerve and we may have seen more meaningful results. It is my recommendation that a well-insulated, lightweight and purpose-built electrode should be used, however, these can be ineffective and expensive (Fulton *et al.*, 2003). The search for suitable device would be valuable, it is important that we can demonstrate that electrostimulation of the first cervical nerve in a NMP graft results in abduction of the left arytenoid. Once this circuit is established

then the effects of the electrostimulation can be evaluated. It is a technique of promise that warrants further investigation but it is vital that the surgical technique of electrode implantation is deliberate and proper. A potentially suitable device may have been identified by a Belgian group (Vanschandevijl *et al.*, 2009). A Cybertronics vagal nerve stimulation electrode (3.00 mm, model 302) was wound around the left recurrent laryngeal nerve and connected to a pulse generator (Cybertronics, model 102), which allowed stimulation parameters to be adjusted using a telemetric wand (Vanschandevijl *et al.*, 2009). Abduction of the left arytenoid cartilage was noted with stimulation frequencies ranging from 2-30 Hz and stimulation intensity of 1 mA (pulse width 250 μ sec during 30 sec). The horse that this stimulation device was implanted in could be stimulated whilst standing and unsedated. A similar device should be tested on the first cervical nerve following a NMP graft procedure.

In this experiment, we tunneled the wires out of the skin at the level of the distal aspect of the proximal third of the cervical region. This was a mistake as it allowed the wires too much exposure and, in hindsight, their damage and loss was unavoidable. More attention should be paid to their location, tunneling and placement under the mandible would be appropriate (Fulton *et al.*, 2003) or maintenance in a tissue pocket may be a solution.

I believe that there is a great deal of potential in the use of electrostimulation of NMP grafts in horses. It has the possibility of providing an affordable and manageable method to improve the technique. Due to our inability to test our hypothesis, this

piece of research cannot provide any real information on the technique for electrostimulation other than to note the technical nuances and difficulties with placing electrodes around the first cervical nerve. In conclusion, I leave you with a quotation:

"Don't be discouraged by a failure. It can be a positive experience. Failure is, in a sense, the highway to success, inasmuch as every discovery of what is false leads us to seek earnestly after what is true, and every fresh experience points out some form of error which we shall afterwards carefully avoid."

John Keats

English lyric poet (1795 - 1821)

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