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Dilation of the oropharynx via selective stimulation of the hypoglossal nerve

Jingtao Huang

NOTE TO USERS

DILATION OF THE OROPHARYNX VIA SELECTIVE STIMULATION OF THE HYPOGLOSSAL NERVE

by

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ABSTRACT

Obstructive sleep apnea (OSA) is caused by the retraction of the tongue to occlude the upper airway (UAW). Electrical stimulation of the tongue protrudor and retractor muscle has been demonstrated as an effective technique to alleviate UAW obstructions and is considered to be a potential treatment for OSA. Recent studies have shown that selective stimulation of the hypoglossal nerve (HG) to activate tongue muscles using a single implantable device presents an attractive approach for treating OSA. In this study, the functional outcome of selective hypoglossal nerve stimulation with a multi-contact peripheral nerve electrode was studied by imaging the airway in anesthetized beagles. A pulse train of varying amplitude was applied through each one of the tripolar contact sets of the nerve electrode while the pharyngeal images were acquired via a video grabber into a computer. For the open mouth positions, the tongue activation patterns were also viewed and videotaped with a digital camcorder through the mouth. The percent dilation of the pharyngeal opening for each contact was calculated. The images show that stimulations delivered through the electrode contacts placed around the HG nerve trunk can generate several different activation patterns of the tongue muscles. Some of these patterns translate into a substantial increase in the oropharyngeal size, while others do not have any effect on the pharynx. The activation patterns vary as a function of the head position and the lower jaw. These results suggest that selective nerve stimulation can be a useful technique to maximize the effects of HG nerve stimulation in removing the obstructions in sleep apnea patients.

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Author Jingtao Huang

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CHAPTER 1

INTRODUCTION/LITERATURE REVIEW

1.1 Anatomy and Physiology

1.1.1 The Pharynx

The pharynx is the passage common to both the respiratory and the digestive systems. The pharynx of a dog consists of nasal, oral, and laryngeal parts. The nasopharynx is the respiratory portion above the soft palate and extends from the choanae of the nasal cavity to the intrapharyngeal opening of the pharynx. The oropharynx is the portion below the soft palate and extends from the isthmus of the fauces to the intrapharyngeal opening dorsally and to the larynx ventrally. The oropharynx is bounded dorsally by the soft palate, ventrally by the root of the tongue, and laterally by the tonsillar fossa with its contained palatine tonsil. Unlike the nasal passage or the larynx, which have cartilaginous support, the pharynx lacks a strong structural support, and thus it is potentially collapsible [1]. The epiglottis resembles a sharp-pointed spade and positions differently during respiration and swallowing. Fig. 1 illustrates the structure of the pharynx.

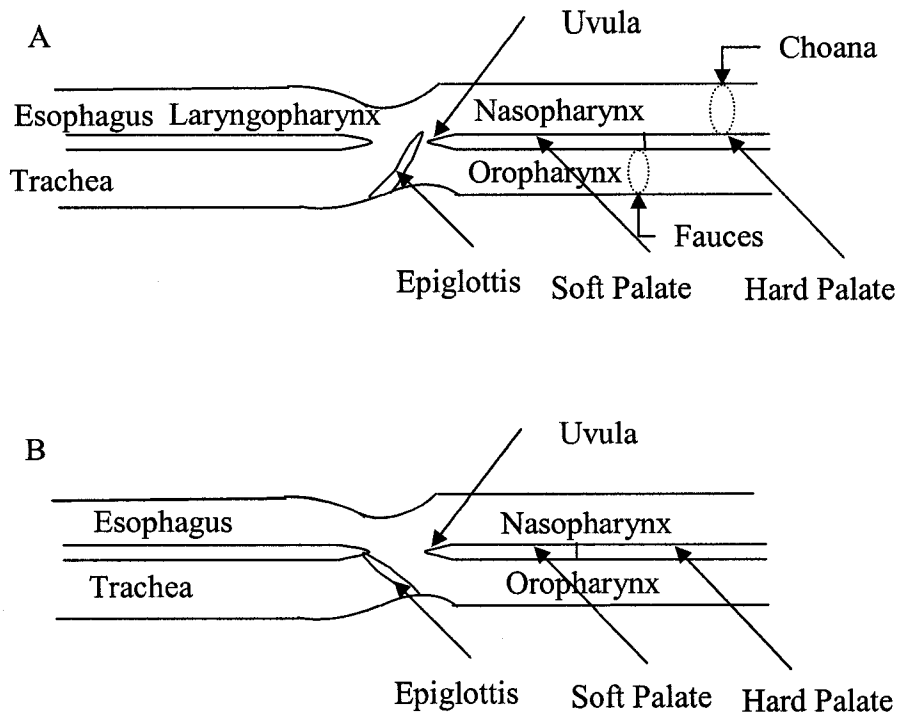

Figure 1. Relation of portions of the pharynx to the esophagus and the trachea. A, during normal respiration. B, during swallowing.

1.1.2 The Hypoglossal Nerve and the Tongue Muscles

The hypoglossal nerve (HG) is a somatic efferent (motor) nerve. Similar to humans [2], the canine HG nerve enters the submandibular region between the mylohyoid and hyoglossus muscles and becomes progressively elongated at the bifurcation site. The HG nerve innervates both intrinsic and extrinsic muscles of the tongue. These muscles include the hyoglossus (HyG), genioglossus (GG), and styloglossus (SG). The geniohyoidus (GH) passing from the mandibular symphysis to the badihyoid bone is also innervated by this nerve. Each GG muscle has bilateral innervation from both sides of the HG nerve [3].

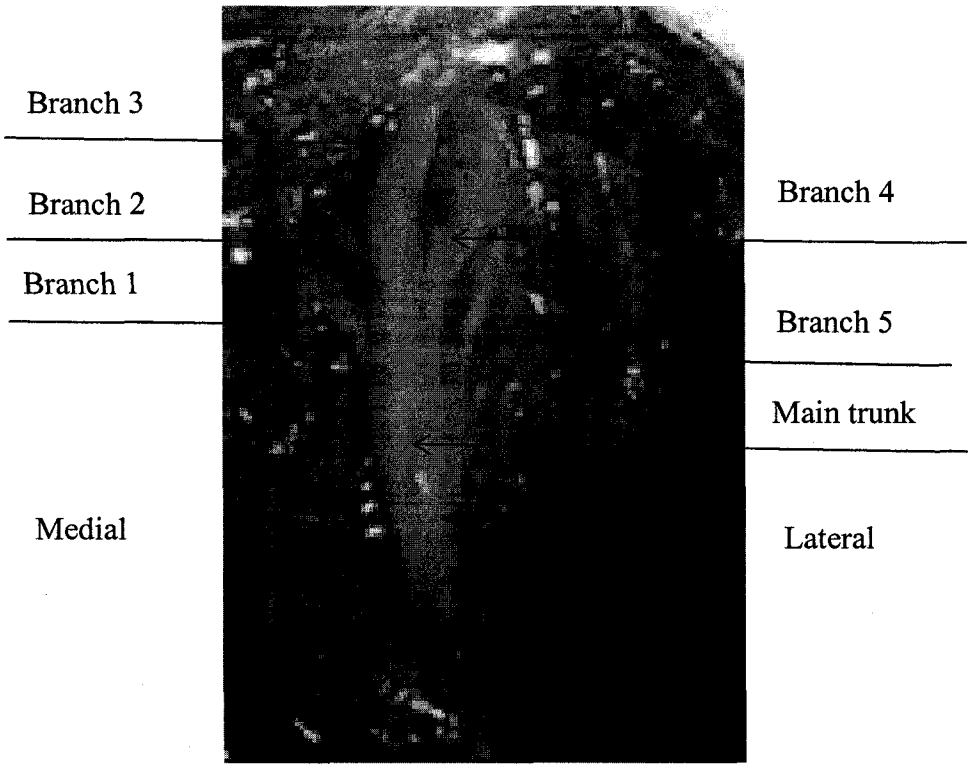

Figure 2. The branching of the left hypoglossal nerve in beagles. The image shows 5 major branches of the left hypoglossal nerve from one beagle experiment.

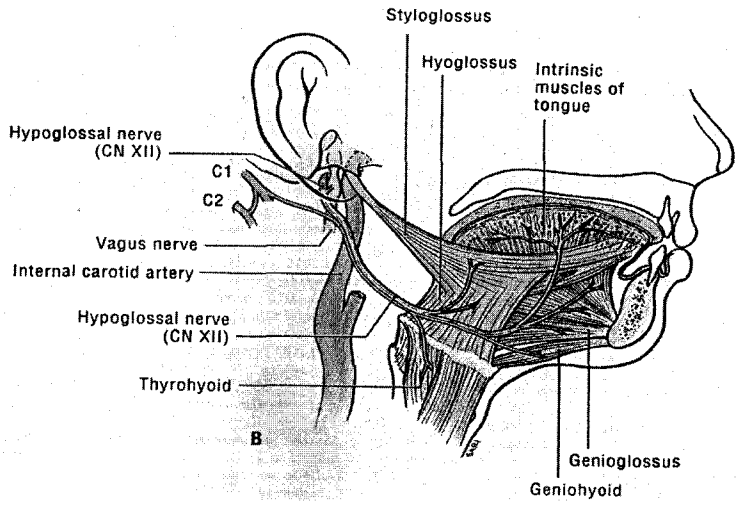

Figure 3. Schematic of the hypoglossal nerve and the tongue muscles in a human.

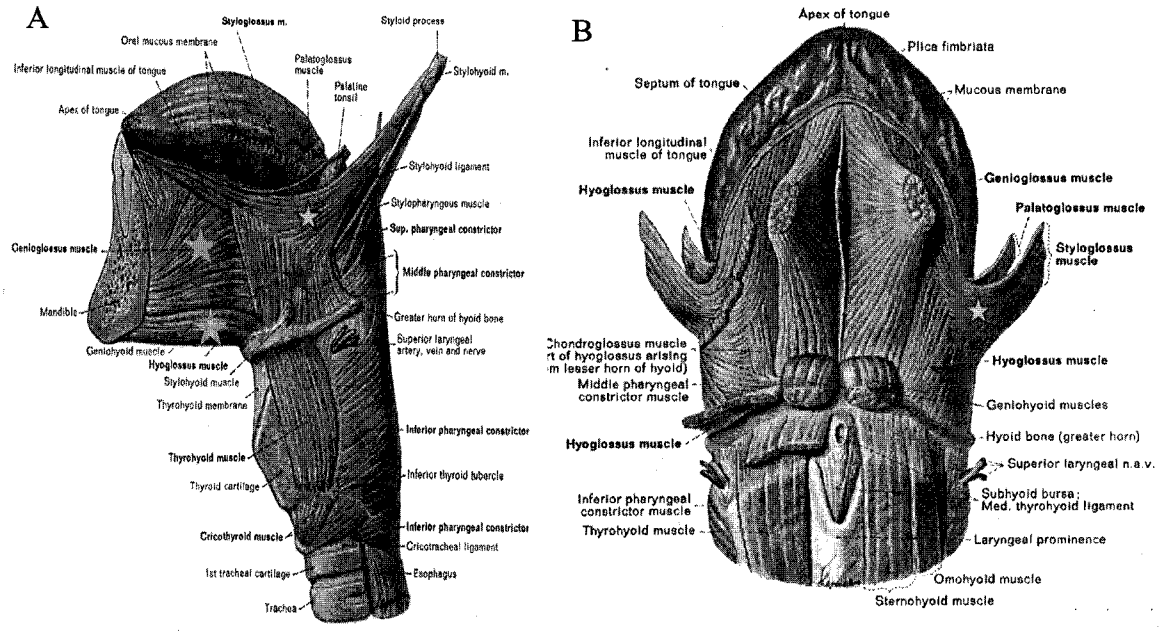

Figure 4. A: The hypoglossal nerve and the muscles innervated by this nerve. B: The tongue muscles are shown from the ventral aspect of the tongue.

The complex but precise movements of the dog's tongue depend on the coordinated actions of its extrinsic and intrinsic muscles. The function of the GG is to depress and protrude the tongue, while the function of the SG and the HyG is to retract the tongue. As a unit, the intrinsic muscles bring about complicated intricate local movement. Also, the function of the GH is to draw the hyoid apparatus cranially and to maintain a patent airway. All of the above muscles are responsible for upper airway patency and innervated by the HG nerve [3]. Activation of the GG muscle has demonstrated a significant increase of the pharyngeal caliber and reduction of the apneic episodes in OSA patients. Activation of the SG and HyG muscles can decrease the upper airway (UAW) compliance, and together with the activation of the GG, they can significantly improve the outcome of GG activation.

1.1.3 The Anatomic Differences Between Humans and Other Mammals

There are at least three anatomic differences in the pharyngeal airway in humans compared with that in mammals that cause the human airway to be more vulnerable to develop upper airway closure during sleep:

1) The abrupt turn of the human pharyngeal airway at the nasopharynx gives it an "L" shape whereas the canine pharynx is a straight tube;

2) In humans, there is an anatomic uncoupling of the epiglottis and soft palate. The tip of the epiglottis is separated from the rim of the soft palate whereas in animals these two structures are at the same cross-sectional level;

3) The hyoid bone has no bony attachments in humans. This floating structure could lead to greater instability of the anterior pharyngeal wall than exists in other mammals in which the hyoid bone articulates with the vertebral column [4].

Despite these anatomic differences, the canine airway was chosen for investigation in the current study as it has a neuromuscular anatomy that is otherwise

1.2 Obstructive Sleep Apnea

1.2.1 Epidemiology

OSA is the intermittent occlusion of the UAW resulting in frequent arousals during sleep [5] (Fig. 5). It is defined as the absence of airflow for more than ten seconds despite continuing ventilatory efforts, five or more times per hour of sleep with an associated decrease in arterial oxygen saturation (SaO_2) of more than 4% [6].

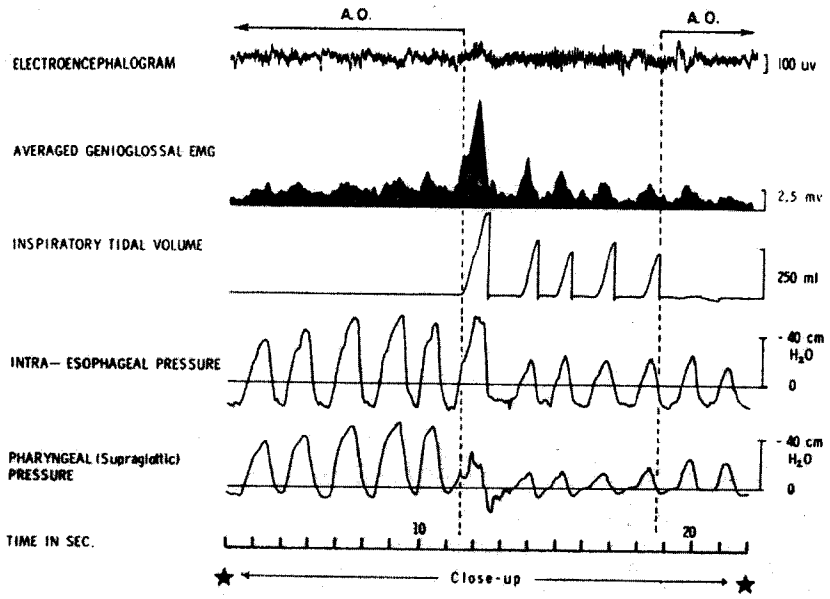

Figure 5. A typical upper airway occlusion in a patient. Although present GG activity is low during the occlusion while the pressure swings in the airways are maximal. There is arousal just prior to the termination of the occlusive phase as indicated by EEG and the inspiration resumes with a large peak in the GG activity. The patient replaces into the occlusions after a few breaths until the next arousal [1].

Obstructive sleep apnea is recognized at an increasing rate as more sleep centers open around the world. It is a prevalent problem among middle-aged overweight males. In most epidemiology studies, the severity of the OSA is rated by the number of obstructive apnea and hypopnea (intermittent within a breath) episodes per hour of sleep (apnea-hypopnea index, AHI). In a recent study, it was estimated that roughly 1 of every 5 adults has at least mild OSA (defined by AHI $\geq$5) and 1 of every 15 has at least moderate OSA (defined by AHI$\geq$15) in white men and women [8]. In Sweden, 1 to 4% of male population suffers from OSA [9]. In a small town in western Australia, at least 8.5% of the men and 4% of the women had evidence of sleep disordered breathing for at least one third of the night [10]. In another study conducted in Australia, the prevalence of sleep-disordered breathing (AHI>15), in a sample of 2,202 subjects between the ages

of 35 to 69, was at least 3.6% (5.7% in men and 1.2% in women) [11]. These statistics demonstrate that OSA is a prevalent sleep disorder in all parts of the world.

Although there is a growing recognition of OSA, there is a disparity between the high prevalence of occult sleep apnea in the general population and the presumed low level of its clinical recognition. The prevalence of clinically diagnosed OSA in middle aged adults showed that at least 80% of all moderate to severe OSA is likely missed [12].

1.2.2 Pathogenesis

The pathogenesis of OSA has been extensively studied and reviewed [1, 13-19]. In brief, along with the secondary variables like excessive weight, gender, age, and the use of drugs that depress the upper airway tone, the current research suggests that the interplay between dilating factors (pharyngeal muscle activation) and collapsing factors (anatomy, airway negative pressure) is the main mechanism for obstructions [20].

Isono and colleagues assessed pharyngeal airway size using endoscopic techniques in patients with apnea and healthy controls under anesthetized conditions and found that patients with apnea have a smaller pharyngeal airway and an increased airway collapsibility compared with controls [21]. Some of the anatomical factors that reduce the size of the air passage are small or recessed lower jaw (micrognathia or retrognathia) [22, 23]. Although OSA patients may have no significant difference in bony structures compared with normal subjects [24], an increase in soft tissue inside the enclosure provided by the bony structure at the level of pharynx may limit the size of the pharyngeal lumen [21].

A sophisticated motor control system including over 20 upper airway muscles is involved in maintaining pharyngeal patency. In healthy people during wakefulness,

pharyngeal patency is carefully protected by these pharyngeal dilator muscles. Researches show that the hypoglossal motor system in the medulla, which controls efferent traffic to the GG, an important pharyngeal dilator muscle of the tongue, can be affected by many variables. Such variables include cortical (behavioral) events, respiratory pattern generating neurons (breathing), peripheral and central chemoreceptors (PaO_2, $PaCO_2$), and input from local mechanoreceptors present in the upper airway itself. Several neurochemical systems (cholinergic, adrenergic, serotonergic, and orexinergic) are also important in the modulation of sleep. Thus, hypoglossal neural activity and thereby genioglossal activation can be precisely modulated to meet the physical demands of the upper airway.

Negative airway pressure (collapsing pressure) is probably the most important local stimulus to the muscles' activation. Even in the absence of central respiratory modulation, these muscles are able to respond within milliseconds to negative pharyngeal pressure, thereby maintaining airway patency [25].

Even in patients with very severe apnea, disordered breathing events occur only during sleep, emphasizing the importance of sleep in the pathogenesis of this disorder. The phasic GG activity in OSA patients was shown to be approximately three times that of the normals during wakened sate to compensate for the anatomic restriction of flow [26]. This compensation is incomplete, though, because the apneic subjects have smaller cross-sectional area of pharynx [27-30] and higher supraglottic resistance during awakened state [31]. The fall in the tonic and phasic activity of the UAW muscles during sleep increases the UAW collapsibility and results in the closure of the pharynx in the face of unfavorable anatomical factors. It has been shown that although the decrease in

the GG activity was not always present [32], GG, lensor palatini, and medial pterygoid muscle activities had significantly larger decrements than controls at the sleep onset [26, 33-36]. As a result, the tongue and the soft palate tend to fall backward [37]. The relatively larger loss in the UAW muscle activity from the already elevated levels renders the UAWs more collapsible than that of normals. The GG activity further decreased during a transition from Non-Rapid Eye Movement (NREM) to Rapid Eye Movement (REM) sleep [38]. The ability of the pharyngeal dilator muscles to respond to negative pressure is substantially attenuated during sleep. Unstable ventilatory control may also contribute to sleep disordered breathing in some patients. Overall, because the negative airway pressure created by the diaphragm muscle during inspiration is not sufficiently balanced by the dilating forces of the UAW muscles, the airways collapse at the most vulnerable sites, the soft palate (nasopharynx and velopharynx) or behind the tongue, i.e. oropharynx [39]. It is worth noting that central apnea differs from obstructive apnea in its origin and form. Since the respiratory drive ceases in central apneas, breathing does not occur even if the upper airways are open.

1.2.3 Symptoms and Current Treatment

Most common complications are excessive daytime sleepiness, restless sleep, morning headache, job-related accidents [40], and impaired short-term memory. Various cardiovascular diseases associated with OSA include hypertension, cardiac arrhythmia, myocardial infarction, and right-sided heart failure. Personality disorder and other psychological problems may further complicate the situation over the long run [41].

In mild cases, the symptoms can significantly be improved by carefully positioning the body before falling sleep, losing weight [42, 43], and avoiding alcohol and sedatives.

Neck extension dilates and stiffens the velo- and oropharyngeal airway, improving the pharyngeal airway [44]. For severe patients, the most common therapies for OSA include tracheostomy, surgical approaches, oral (dental) devices, drug therapy, and continuous positive airway pressure (CPAP).

Tracheostomy or complete bypass of the upper airways is used in persons with severe, life-threatening sleep apnea. Although highly effective, these treatments are extreme procedures that are poorly tolerated by patients and rarely used. Morbidity, including an increased risk for postobstructive pulmonary edema, has been associated with those surgeries [45]. Tracheostomy may also cause stomal infection and accumulation of granulation tissue and it can interfere with speech, exercise, and social interactions. Chronic cough, irritation from cold air, positional pain, and dyspnea (difficult breathing) are also common complaints.

Surgical operations include removing the extra tissue around the uvula (Uvulopalato-pharyngoplasty, or UPPP), radio frequency (RF) ablation of the tongue and mandibular, or tongue advancement. UPPP improves the symptoms in 30 to 50 percent of the cases [46]. Not all patients with OSA are good candidates for surgery because UPPP has been documented to increase the cross-sectional area of the upper airway at the level of the resected soft palate and the uvula [47] while about 50% of patients with OSA has collapse confined to the velopharynx during NREM sleep [47, 48].

At least four types of oral devices are being used, including the soft palate lifter, the tongue-retaining device, the manibular repositioning device, and the tongue posture training device [49]. These devices mechanically increase the oropharyngeal space by advancing the mandible and/or the tongue. The mean AHI is reduced by 56%, and the

compliance range is from 50 to 100% [50]. Known side effects of oral devices include excessive salivation, mouth dryness, or transient discomfort. Long-term problems may include temporomandibular joint or jaw discomfort and movement of teeth [51].

There is no drug therapy that is effective for the long term. Agents studied include ventilatory drive stimulants, central nervous system stimulants, tricyclic antidepressants, serotonin reuptake inhibitors, precursors and antagonists, antihypertensive agents, and even sedative-hypnotic agents [52]. The nonsedating antidepressants protriptyline and fluoxetine reduce the amount of REM sleep time and have been shown to reduce apnea in some patients, but the arousal and arterial oxygen desaturation frequency did not decrease [53]. The progestational agent medroxyprogesterone acetate and the serotonin precursor tryptophan have shown no evidence of improving OSA clinically [54, 55].

The best therapy currently available is a nasal mask that applies a continuous positive airway pressure (CPAP) to keep the airways open. For patients with moderate to severe OSA, CPAP has become the nonsurgical treatment of choice [56]. The CPAP unit typically consists of a self-sealing nasal mask and a compressor, which delivers air under pressure up to 15cm H_2O. The transmural positive pressure is maintained above the critical pressure (Pcrit), the pressure needed to close the UAW, and as a result, apnea events are prevented. While improvements to the original design have reduced the commonly reported side-effects of CPAP (e.g., nasal drying, rhinitis, ear pain, and conjunctivitis), the therapy requires the mask to be worn throughout the night. The low compliance (55 to 70%) of this therapy is a significant issue limiting the use of this device [57].

1.3 Current Approaches to Activate the Tongue Muscles

These reports present a scenario that can potentially be corrected by direct activation of the muscles involved using small electric currents. After all, those individuals with OSA are able to keep their airways open voluntarily during wakened state or resume breathing upon arousal from sleep. The extra dilating forces needed to resume the normal airway patency during sleep can potentially be provided by electrical stimulation of the UAW muscles or the nerves that innervate them. The task then becomes to find the right set of the muscles or the nerves that can dilate the site of obstruction maximally with minimum disturbance to the subject. The sensation due to the electrical stimulation, however, should not even cause micro arousals. Otherwise, the main objective would be defeated by reducing the total amount of time that the patient spends in deep stages of sleep.

1.3.1 Genioglossal Stimulation

A study conducted in nine OSA patients demonstrated that unilateral GG stimulation with acutely implanted wire electrodes can increase the inspiratory airflow at moderate levels of CPAP without arousal from sleep [58]. The GG activation increased the maximum inspiratory flow rates significantly (although the flow limitation was not completely abolished) while the retractor muscle (HyG and SG) stimulation decreased it. The repetitive GG stimulation in four of those patients decreased the AHI from 65.6±11.5 to 9.0±5.8 episodes/h. In another study, percutaneously inserted bipolar hooked wires successfully increased the diameter of the hypopharyngeal airway up to 284% during wakened state in nine of the 14 patients studied [59]. In seven awake healthy subjects, the UAWs were partially occluded by applying external pressure to the

submental hyoid region [60]. Transmucosal stimulation of the tongue base, which presumably activated the GG, effectively reduced (about 42%) the pharyngeal resistance, despite the fact that submental stimulation (see below) did not generate any statistically significant changes. Another group tested the effects of direct GG stimulation on UAW resistance in anesthetized dogs [61]. Upper airway resistance (Rua) increased during both inspiration and expiration when the tracheal negative pressure was increased from 5 to 20 cm H_2O. Airway resistance was significantly reduced by stimulation of the GG at either tracheal negative pressure. The effect of stimulation on airway resistance decreased remarkably with the stimulation frequency and reached a plateau at around 50 Hz.

Direct activation of GG with wire electrodes cannot become a method of choice to be used at home on a daily basis for obvious reasons. However, these reports suggest that the GG activation alone is capable of improving the airway patency. A concern in these studies is the placement of the wire electrodes inside the GG muscle. The effect of stimulation may vary significantly depending on the site of implantation.

1.3.2 Submental Stimulation

It is the GG muscle again that is targeted with transcutaneous stimulations using electrodes placed underneath the mandible, the lower jaw. Miki et al. [62] examined the effects of submental electrical stimulation in six patients. The stimulations decreased the frequency of apneic episodes, apnea time/total sleep time, the longest apnea duration, and the number of times that oxygen saturation dropped below 85% per hour significantly compared to those with controlled nights. Stimulation did not cause arousals or affect blood pressure or heart rate significantly. Hida et al. reported that

submental stimulation in thirteen patients reduced the frequency and duration of apneic episodes with an improvement in the sleep quality and daytime sleepiness [63]. These effects remained for at least two nights following the five successive stimulation nights. None of the patients was awakened by the stimulation and none complained of pain or any other discomfort due to stimulations. Another report by this group demonstrated that the effect of submental stimulation on upper airway collapsibility were similar to those of hypoglossal nerve stimulation in anesthetized dogs [64], which was to decrease the collapsibility and expand the UAW size.

A controversial study was reported by Edmonds et al. [65] that submental and subhyoidal transcutaneous electrical stimulation in eight male patients with OSA failed to prevent sleep-disordered breathing or improve sleep architecture. Transcutaneous stimulation failed to enlarge the upper airway during wakened state as well as prevent upper airway from collapse during sleep. Decker et al. [66] reported that submental stimulation had inconsistent effects in seven OSA patients, terminating only 22% of the apneas. The submental stimulation was discomforting during wakened state. However, the stimulus intensity that produced arousal during sleep was significantly greater than that producing barely tolerable discomfort while awake. Schnall and his colleagues [60] tested the dilatory effects of the upper airway muscle contraction induced by transcutaneous electrical stimulation in awake subjects. Only sublingual stimulation produced measurable tongue protrusion, which was believed to be the effect of GG activation, and it helped to preserve the upper airway patency in the face of exogenously applied pressure load. Neither submental (geniohyoid) nor paralaryngeal (sternohyoid

and sternothyroid muscles), no matter alone or combined, could cause any tongue protrusion.

In summary, the reports on submental stimulation are controversial, which can be attributed to nonspecific activation of GG due to the tissue present between the stimulator and the target muscle. The electrode size and the position can play a significant role on the muscle recruitment function. The submental approach is attractive because of its non-invasiveness and ease of application. The size of the patient population who can benefit from this approach remains to be determined in a larger scale study.

1.3.3 Hypoglossal Nerve Stimulation

Electrical nerve stimulation has a number of advantages over muscle stimulation. Electrode interface is much more stable mechanically during activation and therefore the recruitment characteristics are better defined. Neural stimulation requires much less energy than muscle stimulation [67]. Thus, HG nerve stimulation is preferred over GG activation if the same function can be achieved. The hypoglossal nerve is mainly a motor nerve. It is not known exactly how many sensory fibers exist in the human HG nerve; however, the experience in clinical trials demonstrate that HG nerve stimulation at moderate levels does not cause pain to the subject and the threshold for arousal is even higher during sleep [66].

Direct hypoglossal nerve stimulation in UAW isolated dogs caused a remarkable decrease of upper airway compliance [64], defined as the slope of the pressure-volume (P-V) curve. With chronic implants in dogs, it has been shown that unilateral hypoglossal nerve stimulation can increase the peak upper airway flow from 0.1 L/s to

1.6 L/s tested over a 3-month period [68]. Histology examination revealed no nerve damage resulted from chronic stimulation. In humans with intra-operative acute nerve cuff implants on the HG nerve, the flow of inspired air was doubled by stimulation of the main trunk of the HG nerve [69]. Stimulation of the medial branch was nearly as good as that of the main trunk and was superior to stimulation of other branches. Stimulation of the distal HG nerve to the GG caused protrusion and contralateral deviation of the tongue [70]. Hypoglossal nerve stimulation at both loci during sleep consistently resulted in an increased inspiratory airflow without arousal from sleep.

Decker et al. reported [66] that the HG nerve stimulation with percutaneously inserted wire electrodes provided tongue protrusion at minimal discomfort in humans; yet it terminated only 23% of the apneic events. Stimulation with bipolar needle electrodes by another group was shown to interrupt obstructions in human subjects without arousals [71]. As was discussed by the former group, the inefficiency of the stimulations with wire electrodes could be due to the inappropriate placement of the electrodes resulting in the recruitment of refractory muscles before the protrudor muscles of the tongue.

Individual and combined stimulation of the HG nerve branches caused the greatest increase in the airway area in the rostral oropharyngeal airway, though the area also increased significantly in the caudal oropharynx [4].

Coactivation of the lateral HG nerve branches (HyG and SG) and the medial branches (GG) has been investigated. The results showed that the added effects of HyG (tongue depression and retraction) and SG (retraction and elevation of lateral aspects of

tongue) muscles improved the outcome of GG activation with an increased airflow rate and the mechanical UAW stability with the Pcrit decreased significantly [4].

The most advanced efforts on HG stimulation has been led by a group at Johns Hopkins University and their collaborators from various other research centers and Medtronics Inc., MN. In an international collaboration effort, this group chronically implanted eight OSA patients with a device that stimulated the hypoglossal nerve unilaterally [72]. The device consisted of an implantable intra-thoracic pressure sensor for synchronization with breathing, a programmable pulse-generating device, and a stimulating half cuff nerve electrode (Fig. 6) placed around the medial branch of the HG nerve. Electrical stimulation was delivered for the entire night after the onset of the sleep and significantly reduced the mean apnea-hypopnea indices in NREM and REM sleep stages, and it reduced the severity of oxyhemoglobin desaturation. The long-term stimulation at night was tolerated by all the patients without any adverse effects. Although apnea was eliminated entirely in patients, the intermittent inspiratory flow limitation (snoring) remained. The stimulation seemed to be most effective for patients with retroglossal obstruction. Poor synchronization, electrode breakage, and sensor malfunction prevented the continuation of the study in some patients.

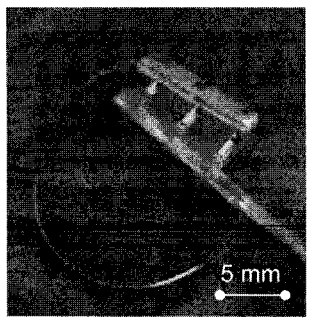

Figure 6. The half cuff neural electrode with tripolar contacts developed by Medtronic Inc. (Model 3990) for stimulation of HG nerve in OSA patients.

In summary, the HG nerve stimulation has been demonstrated to be a promising treatment method for OSA. Different rates of success in the reports are most likely due to the variations between the subjects and the site of electrode implantation. There are still improvements needed before this approach can become a clinical tool. One of these techniques, selective nerve stimulation, is discussed below.

1.3.4 Selective Stimulation of the Hypoglossal Nerve

To reduce the number of implanted electrodes for hypoglossal nerve stimulation, electrodes are required to be able to stimulate independently several muscles [73]. Selective stimulation of individual components of a peripheral nerve with multiple fascicles would allow the control of a large number of muscles with less implanted hardware. This technique was investigated as a method to improve the functional outcome of the HG nerve stimulation [74, 75].

Studies have shown that in the distal portion of the nerve trunks, most axons will be arranged in fascicles (i.e. bundles) that innervate only a single muscle. In the proximal portion of a nerve trunk, intermixing of fascicles results in a more homogeneous arrangement of axons with respect to their end-organ innervations [76]. These studies imply that potential peripheral nerve implant sites will exist where fascicles are not greatly intermixed. In these cases, the electrical stimulation of a single fascicle will result in selective activation of one muscle.

1.3.4.1 Cuff Electrode There are several kinds of electrodes under development for selective stimulation of peripheral nerves including intraneural wire and silicon electrodes, intrafascicular electrodes, epineural electrodes, and multiple contact nerve cuff electrodes [77].

McNeal and Bowman [78] have demonstrated that with a proper fit and positioning, a single circumneural sleeve with multiple electrode contacts could selectively activate two antagonist muscle groups innervated by a common nerve trunk. Selective activation of individual peripheral nerve fascicles has been achieved with multi-contact spiral nerve cuff electrodes or carefully chosen electrode positions. Even without prior reference to nerve fascicle location, a multiple contact extraneural cuff allowed selective and independent activation of multiple fascicles within a large nerve trunk [79].

A cuff electrode directly makes contact with a peripheral nerve and is held in place with a compliant sheath that is wrapped around the nerve. Conventional multi-contact cuff electrode with round cross-sectional geometries [77] have shown significant advances in selectivity. The features that make the nerve cuffs more attractive than other types of electrodes are [80]:

1) For motor prostheses, muscle length and limb position have little or no effect on the recruitment characteristics of cuff electrodes, unlike surface and muscle electrodes.

2) The stimulus magnitude required for nerve activation is minimized with nerve electrodes, minimizing the likelihood of electrically-induced tissue destruction and conserving stimulator power.

3) Cuffs may be positioned so that relative motion caused by muscle contraction and limb movement is minimized, resulting in the longer lifetime for the electrode.

4) The excitatory field within a cuff can be accurately controlled, and it is possible to precisely manipulate current flow and regulate the particular neural elements that are activated or blocked.

5) It is possible to block neural conduction and activate the motor units in their natural recruitment order with nerve cuff electrodes.

1.3.4.2 Other Electrode Designs There are also some novel electrode designs with significant advances in selectivity. The Utah slanted electrode has been shown to be able to achieve a broader recruitment curve than cuff electrodes, which means higher muscle group selectivity. Also, it can activate small sub-populations of fibers within a single fascicle [67]. Intrafascicular electrodes must penetrate the epineurium surrounding the entire nerve and the perineurium that surrounds each fascicle. The Utah Slanted Electrode Array, or USEA, has a 10×10 array of electrodes with varying lengths (0.5-1.5 mm) at 400 μm spacing. The electrodes are insulated with silicon nitride and only the platinum-plated tips are exposed with an area of about 0.005mm^2. Subfascicular selectivity can be achieved using USEA in acute and chronic experiments, but the implantation is an invasive process that can potentially cause significant damage to the nerve because the physical barrier of the nerve fibers, perineurium, was penetrated. It has been shown that surgical techniques highly affected the long-term results of Utah electrodes. Moreover, movement of the surrounding muscles can displace the array, causing damage to the nerve. Problems related to the electrode interface and its chronic implantation have not been solved [81].

1.3.4.3 FINE A Flat Interface Nerve Electrode (FINE) with multiple contacts [82] was used for selectively activating various fascicles inside the HG nerve trunk (Fig. 7). The FINE works on the principle that a peripheral nerve can be reshaped slowly without introducing trauma [59]. It is designed to reshape the nerve into a configuration that moves the axons closer to the stimulating contacts and increases the nerve surface area

[82]. It allows more stimulation contacts to be placed around a nerve and minimizes the distances from any given axon to a stimulation contact. FINE with 9 contacts on the proximal hypoglossal nerve trunk was implanted in a dog [74]. The data showed that genioglossus or geniohyoid can selectively be activated from the main HG trunk by delivering small currents through selected contacts. Selective stimulation of the HG nerve with A 12-contact FINE (Fig. 8) implanted immediately proximal to the branching point showed that the protrusor (GG) and retractor muscles (HyG and SG) can be activated selectively [83]. However, subfascicular selectivity or selective activation of styloglossus and hyoglossus muscles separately was not possible. This may be because the nerve fibers innervating each of these muscles are cross-mingled between the fascicles in the HG nerve.

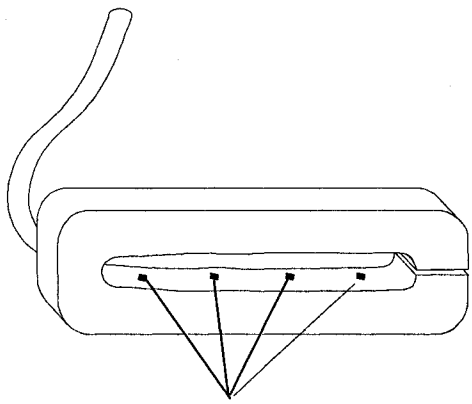

Figure 7. The Flat Interface Nerve Electrode (FINE).

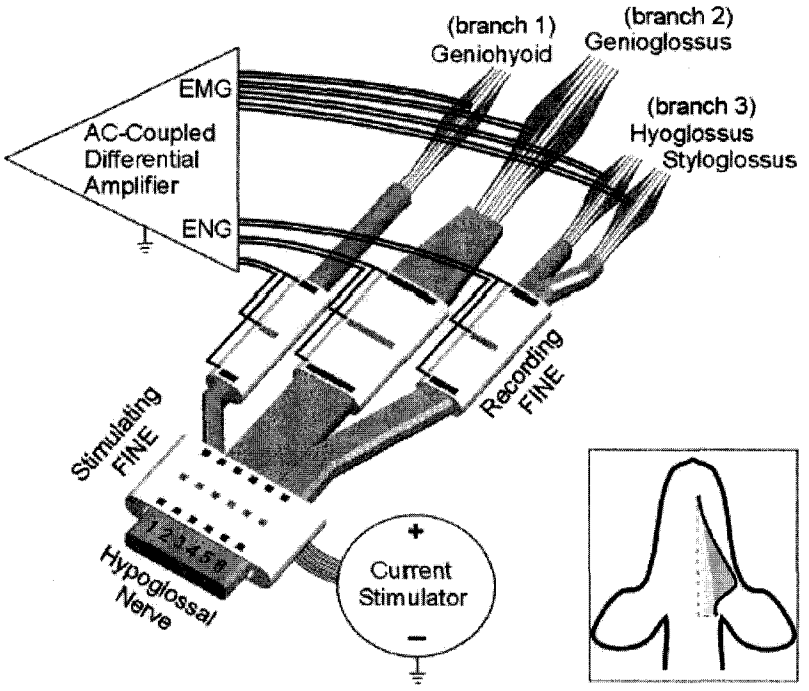

Figure 8. Placement of the Flat Interface Nerve Electrode on the hypoglossal nerve for selective stimulation [83].

1.4 Imaging of the Upper Airway

Knowledge of the morphology and mechanical behavior of the upper airway is essential for a more complete understanding of OSA. Computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and endoscopy have been used to measure the caliber of the upper airway with or without stimulation of the muscle or the HG nerve [84].

- CT is relatively expensive but widely available and able to provide an accurate assessment of the upper airway cross-sectional area and volume. Images can only be acquired in the axial plane. Radiation exposure limits the ability to perform repeat studies during the awakened state of sleep.

- With MRI, it is possible to assess the upper airway caliber without radiation and it is good for soft-tissue characterization. Direct sagittal, coronal and axial images can be acquired without radiation. The shortcoming of this technique is its high cost.
- Ultrasound is noninvasive without radiation and easily repeated. It is performed through the mouth, which alters the upper airway anatomy. Moreover, it does not provide a high-resolution anatomic representation of the airway or soft-tissue structures.
- Endoscopy is widely available and easy to perform without radiation. It evaluates only the airway lumen, not surrounding the soft-tissue structures.

In this study, the endoscopy method was used to assess the dilation of oropharynx during stimulation.

1.5 Research Hypothesis

The main objective of this study was to investigate tongue activation patterns and pharyngeal opening during electrical stimulation of the HG nerve with a multi-contact cuff electrode. The image recording and electrical stimulation techniques of this study were developed with the ultimate goal to implant a single neuroprosthetic device in human patients.

Three main hypotheses are tested in this study:

Hypothesis 1: A multi-contact nerve cuff electrode can cause different tongue activation patterns.

This hypothesis was tested in anesthetized beagles, where the tongue movements were recorded at different head positions with the mouth open during electrical

stimulation when different amplitudes were applied to each contact of the FINE electrode.

Hypothesis 2: Depression of the tongue base is associated with pharyngeal opening.

This hypothesis was tested in acute beagle experiments. The tongue movement images were compared with the corresponding pharyngeal images.

Hypothesis 3: Selective HG stimulation can cause comparable pharyngeal opening to that of the HG branch stimulation.

This hypothesis was tested by comparing pharyngeal images during FINE and branch stimulation obtained in the acute beagle experiments.

CHAPTER 2

METHODOLOGY

2.1 Stimulation Wave Form

The pulse form of the electrical stimulation is biphasic (Fig. 9). Each pulse consisted of a cathodic phase of 100 μs followed by 100 μs delay and an anodic phase of 400 μs with ¼ amplitude of the cathodic phase.

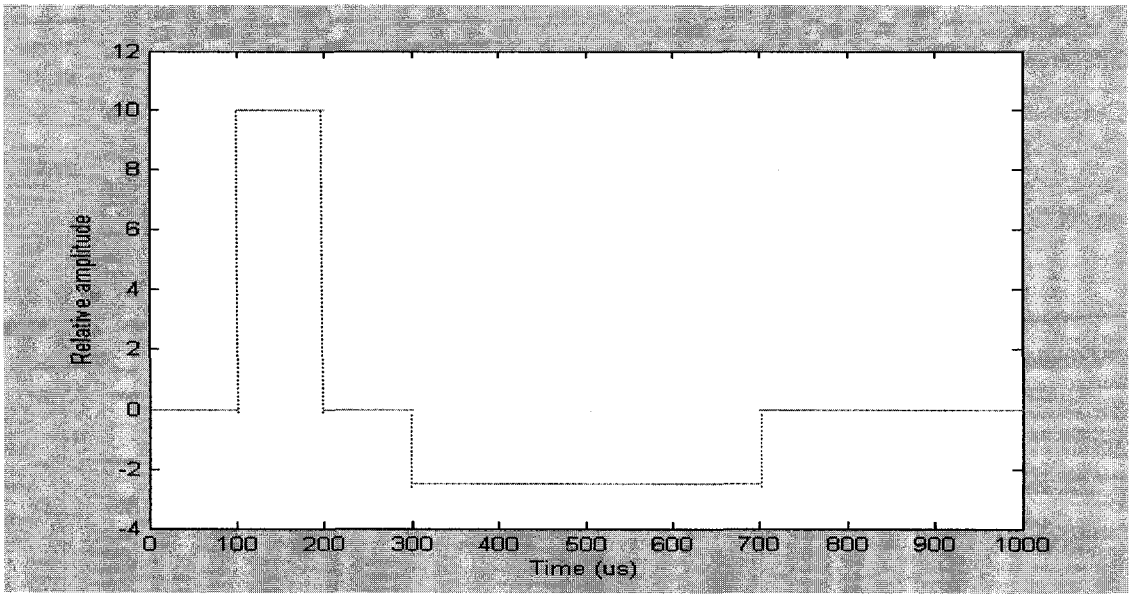

Figure 9. The biphasic pulse of electrical stimulation.

2.2 LabView Program to Test Threshold

The aim of this program is to apply an electrical pulse train through one specific contact to the HG nerve for the viewers to judge if there is visible tongue or pharynx

movement. The pulse frequency is 50 Hz, the cathodic phase of each pulse is 100 μs, and the whole pulse train duration is 1 second.

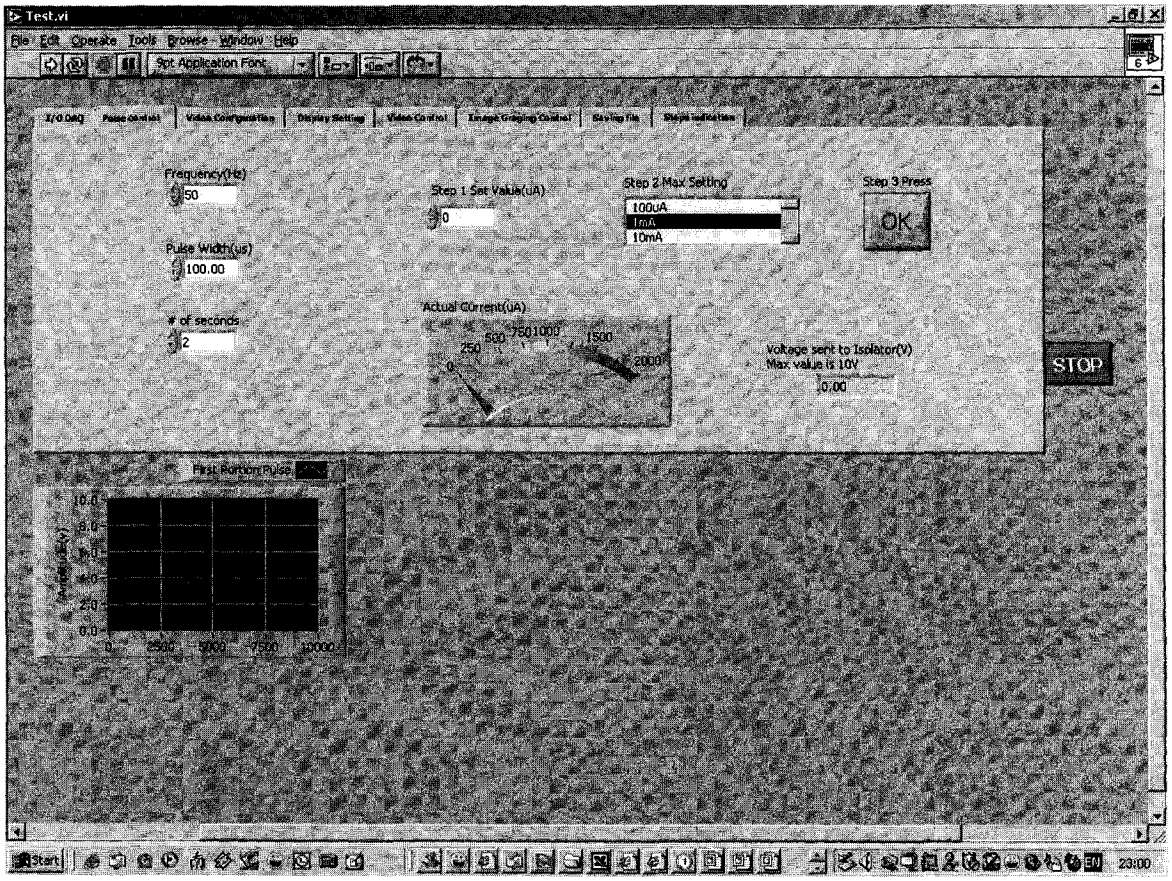

Figure 10. The LabView™ program for testing threshold.

2.3 LabView Program to Apply Electrical Stimulation

The aim of the LabView™ program is to generate pulses with adjustable amplitude, frequency, pulse width and pulse duration to stimulate left HG nerve through one specific contact of FINE and cuff electrodes, and to grab reference and stimulation images in the middle of the pulse train. Fig.12 shows the flow chart of the program.

Step 1. Initialize the Data Acquisition Board. The minimum current level I_{min} and the maximal current level I_{max} were chosen based on the previous threshold test

procedure. The step DI was chosen to make sure there were 5 to 6 current levels for each contact.

Step 2. Grab one reference image without electrical stimulation as a reference. The number of loops N was set to 1 at this time, which means the coming loop would be the first loop. The possible maximal value of N in this study was 5, which means for each contact at each current level the stimulation and image grabbing process would be repeated for 5 times. The image will be named as contact number_current level number_0. For example, contact5_1_0.bmp, which means this image was taken before the electrical stimulation at the first current level which is the threshold level, was applied through contact 5. This image was a reference. Delay 1 is between taking the reference image and the first pulse train. Delay 1 needed to be at least 1500 μs to allow the system to finish image grabbing and generate the pulse trains.

Step 3. LabView program generated the pulse trains at the first current level. The frequency was 50 Hz, pulse width was 100 μs, and the pulse duration was 2 seconds.

Step 4. After 1 second of the beginning of each pulse train, i.e. right in the middle of one pulse train, one image was grabbed and saved as a stimulation image. This process was repeated 5 times for each current level and between 2 pulse trains; there was delay 2, which was 1 second to let the tongue muscles relax to resting state.

The current level would be increased by DI and steps 2 through 4 would be repeated until the current level reached Imax. Then the investigator could write necessary comments to be saved and all the parameters of the pulses would also be saved.

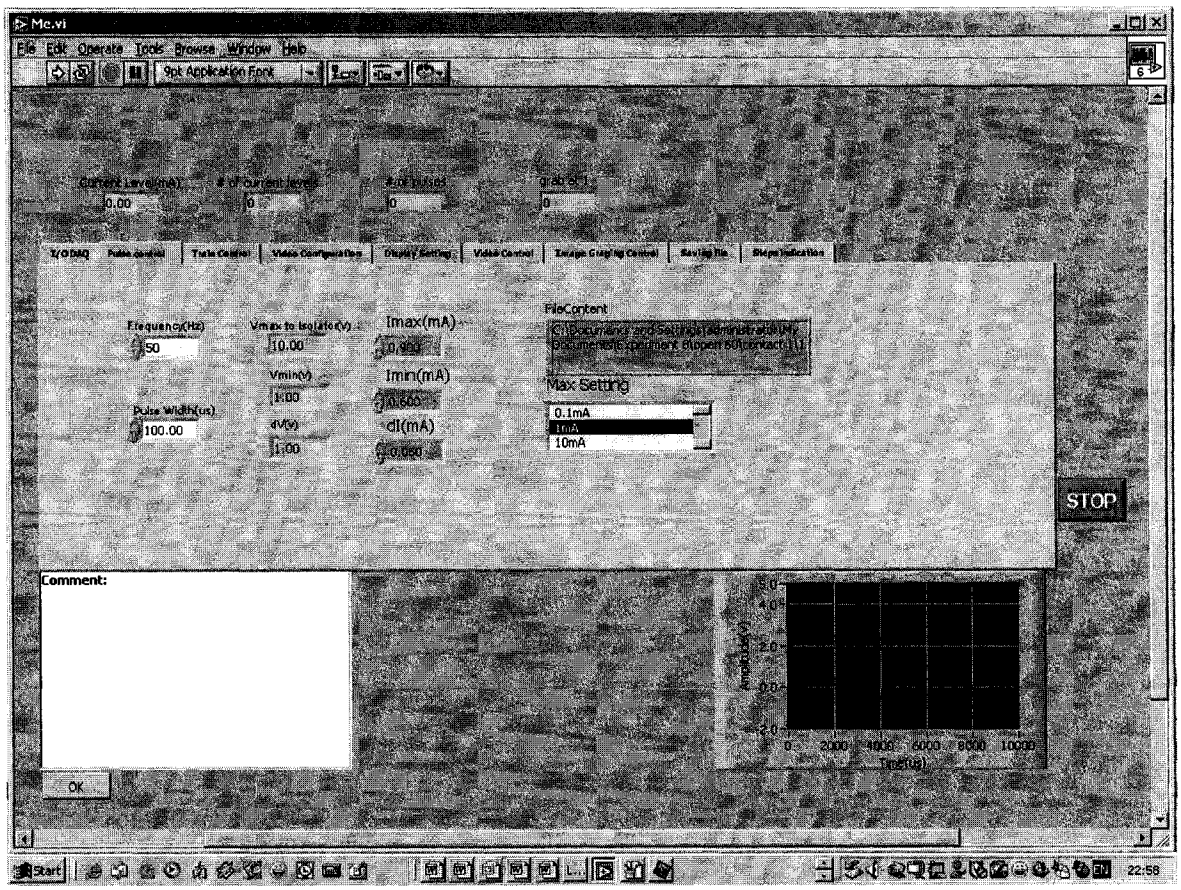

Figure 11. The LabView™ program for applying stimulation and acquiring images.

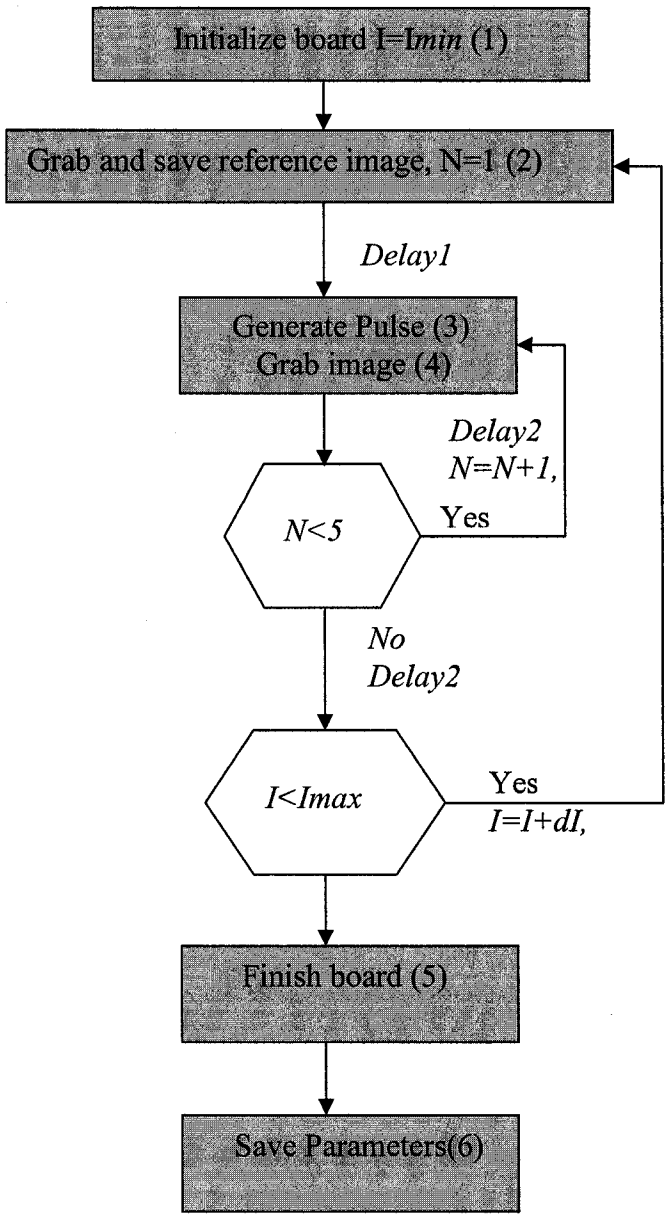

Figure 12. The flowchart of the LabView™ program for applying stimulation and grabbing images.

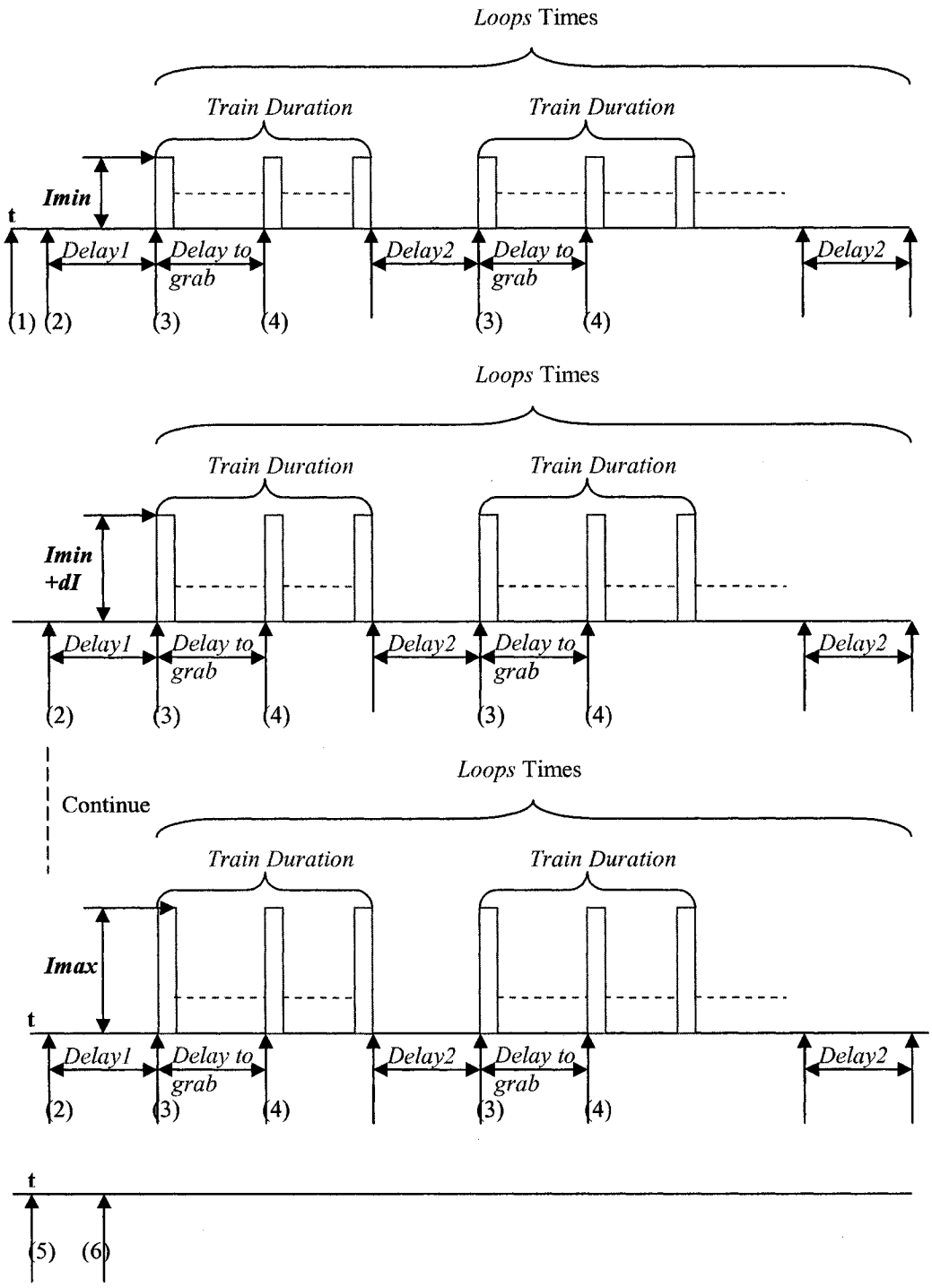

Figure 13. The figure shows the delays and incensement of stimulus pulses in the LabView program.

2.4 Experimental Setup

Experimental protocols were reviewed and approved by the Animal Care and Use Committee at Louisiana Tech University prior to the procedures.

Seven beagles (10 kg) were used in this study. Anesthesia was induced with sodium phentobarbital (30 mg/kg, IV) and maintained with smaller doses (6 mg/kg, IV) when needed. Adequate depth of anesthesia was periodically assessed by the heart rate and absence of the lateral corneal reflex. Dexamethasone (0.25mg/kg) was given to prevent edema at the start of surgery. Surgical areas were shaved and the animal was transferred to a heated-top surgery table. Rectal temperature was kept at 38°C. Femoral artery and vein were catheterized for monitoring blood pressure and injection of fluids. Lactated Ringer's solution was administered intravenously. Tracheotomy was performed and the animal was connected to a mechanical ventilator through the tracheal tube to eliminate spontaneous breathing. End-tidal CO_2 (<4%) and electrocardiogram were monitored.

A longitudinal incision between the midline and the lower jaw bone was made in the submandibular region on the left side. The hypoglossal (HG) nerve was exposed with blunt dissection. A Flat Interface Nerve Electrode (FINE) was implanted on the left hypoglossal nerve trunk immediately proximal to the bifurcation point of the distal branches occurring over the hyoglossus (Fig. 15). The implantation was done by placing the HG nerve into the electrode through the open side of the electrode and then closed with a suture. During the implantation procedure, no effort was made to reshape the nerve by force. The electrode reshaped the nerve over the next few hours because of the elastic properties of the cuff material. Electrodes were implanted without prior

knowledge of the nerve fascicle locations inside the nerve. The lead wires were connected to the stimulator through a switch box.

Cylindrical cuff electrodes were implanted onto individual branches of the HG nerve distal to the FINE. The animal's head was fixed at an angle of either 30 or 60 degrees from the horizontal with the mouth open or closed (Fig. 14). In open mouth cases, the lower jaw and the tongue were pulled up to the vertical position using rubber bands for imaging the tongue and the pharyngeal opening at its root. The tip of the tongue was mechanically stabilized.

A fiberscope lead (Small Animal Bronchoscope 60001VL, diam. 5 mm, KARL STORZ, Charlton, MA) was inserted rostrally through the tracheostomy hole and fixed past the edge of the trachea to image the oro- and naso-pharyngeal openings (Fig. 14). A suture was tied to the epiglottis and pulled into the trachea caudally to clear the opening in front of the fiberscope lead. Various fiberscope tip angles were tried for best viewing.

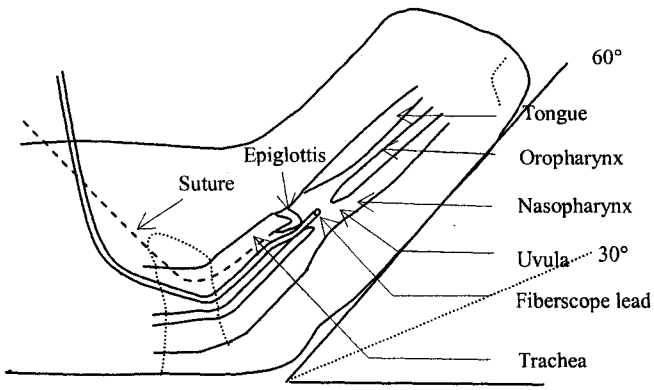

Figure 14. Positioning of the dog's head in 30 and 60 degrees with respect to the horizontal plane and positioning of the fiberscope lead through the tracheal hole before the soft palate.

2.5 Stimulation and Recording Protocol

The electrodes of this study were fabricated using gold contacts deposited on a polyimide substrate. The contacts were molded into the geometry shown in Fig. 15 using a silicone elastomer (MED4-4211, Nusil Silicon Technology, CA). The electrode had 16 sets of tripolar contacts positioned around the nerve trunk, 8 on the top and 8 on the bottom side of the nerve, and numbered sequentially around the nerve in the clockwise direction, viewed from the proximal side of the HG nerve, as shown in Fig. 18B. The cathodes were evenly spaced (inter-contact distance = 1 mm), and the contacts on the top and the bottom part of the electrode had separate anodes on each side of the contacts. The electrode had a window size of 8 mm by 0.6 mm. The cathodic contacts had an area of 1x 0.4 mm. The thickness of the wall was set to 2 mm to provide sufficient force for reshaping the nerve within a few hours.

The custom-made virtual instrument in LabView® (National Instruments) mentioned in Section 2.3, a data acquisition card (NI 6071E, National Instruments), and a linear stimulus isolator (A395, World Precision Instruments) were used to generate the stimulation train.

A train of biphasic current pulses (frequency=50 Hz, train duration=2 s) of varying amplitude (0-2mA) mentioned in Section 2.1 was applied through each of the 16 tripolar contact sets of the nerve electrode using a switch box. Each pulse consisted of a cathodic phase of 100 μs followed by a 100 μs delay and an anodic phase of 400 μs with ¼ amplitude of the cathodic phase.

The threshold for each contact was defined as the minimal current level needed to obtain visible movements of the tongue. Once the threshold for a given contact was

determined, the images were acquired at the activation threshold and 5 suprathreshold levels of increasing amplitudes.

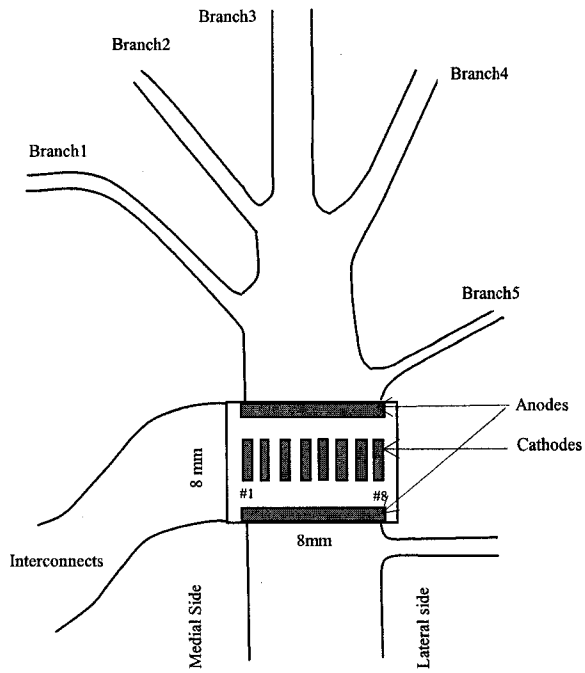

Figure 15. Implantation site of the FINE and branching of the HG nerve (left side). Five major branches are numbered in sequence mediolaterally.

The pharyngeal images were acquired into a computer using the FlashBus MV Lite (Integral Technologies, Inc. Indianapolis, IN) image grabber system: one frame before stimulation as a control and one frame a second after the onset of the stimulus train. The stimulus train was turned off after 2 seconds and the procedure was repeated 5 times for each current level. For the open mouth positions, the tongue activation patterns were also viewed and recorded with a digital camcorder through the mouth along with the fiberoscopy images.

2.6 Data Analysis

Images were loaded into Matlab®, and the pixels surrounding the pharyngeal opening were marked manually. A Matlab® program calculated the area of the cross section

enclosed by the selected pixels. For every contact, one control image and 5 stimulated images (one for each current level) were selected for analysis. The increase in the opening of the oropharynx corresponding to the stimulus level 10% above the threshold was calculated by interpolation. For each contact, the percent dilation in the oropharyngeal opening was found using the following equation:

$$Percent\ Dilation = \frac{A_{stim}}{(A_{stim})_{max}} \times 100 \qquad (1)$$

where A_{stim} represents the oropharyngeal area during stimulation. The maximal value of A_{stim} among all 16 contacts was denoted as $(A_{stim})_{max}$.

The percentage opening with the best contact was compared with the best branch stimulation using

$$Contact\ vs.\ Branch = \frac{A_{contact}}{A_{branch}} \times 100 \qquad (2)$$

where $A_{contact}$ represents the maximal oropharyngeal area from contact stimulation and A_{branch} from branch stimulation.

2.7 Histology

The reshaped section of the left HG nerve was explanted, and it was removed and fixed in 4% buffered paraformaldehyde solution for at least 48 hours. Then the nerve tissue was dehydrated in graded ethanols and embedded in paraffin with anatomic orientations preserved. Twenty-micron thick sections were made and stained with hematoxylin and eosin stain.

CHAPTER 3

RESULTS

3.1 Patterns of Activation in the Tongue and Pharyngeal Images

Transoral images taken with the camcorder demonstrated a number of different modes of tongue movements. Fig. 16 shows some typical movement patterns of the tongue in a 30 degree head position during an open mouth case (see Fig. 17A top left for the names of structures in the images). Medial or lateral depression of the tongue base is observed in these images. Some of the tongue movement patterns were associated with oropharyngeal dilation.

All images of Fig. 16 were taken for the stimulation current levels that were less than 20% suprathreshold for muscle activation. In other experiments, some of the movement modes shown in Fig. 16 were not observed, e.g. simultaneous contraction of both the medial and lateral tongue root (middle image on left). However, contractions of the medial or lateral tongue root were observed through different contacts. When the current level was just above the threshold, only the intrinsic muscle contractions resulting in changes of the tongue's shape were observed. For some contacts, usually the ones on the medial side, when stimulation current was just above threshold, closure of the retropalatal area could be observed (middle image on right). However, the closure

did not translate into a decrease in the oropharyngeal opening, as determined by fiberscopy images. At current levels 10% above the threshold, the activation pattern was always an opening of the oropharynx if there is a change. When the current level was increased further, the movement pattern changed as a result of activating all muscles involved which further increased the retropalatal opening.

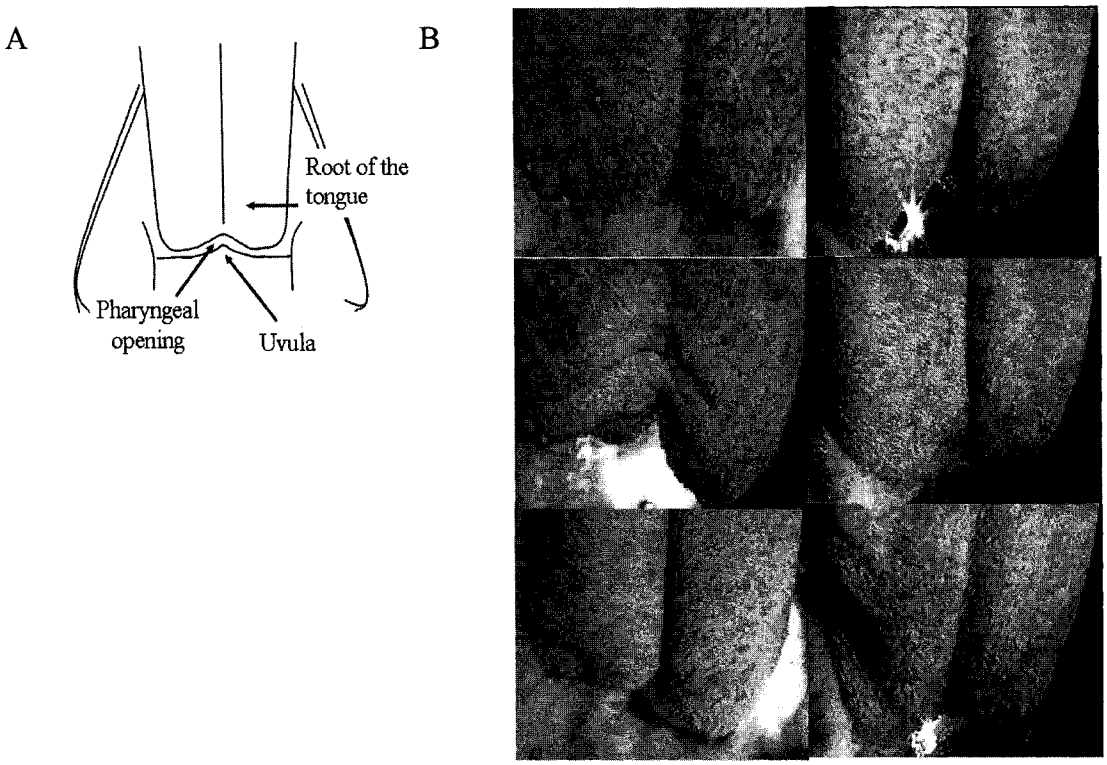

Figure 16. A: The important structures in the tongue images. B: Transoral images of the tongue at rest and during FINE stimulation. The head was 30° from horizontal with the mouth open. The top left image: resting state; top right: contraction of the tongue root in the middle and moderate dilation of the pharynx; middle left: both medial and lateral contractions of the tongue root and a large opening of the pharynx; middle right: closure of the pharynx; bottom left: lateral depression of the tongue without pharyngeal opening; bottom right: lateral depression with some pharyngeal opening due to activation of intrinsic muscles.

3.2 Stimulation through Medial vs. Lateral Contacts

Images taken in 30° head position and an open mouth case in one of the beagles were selected to show typical movement modes of the tongue and the corresponding

pharyngeal images observed in the same experiment (Fig. 17). The activation pattern shifted in the medio-lateral direction when the stimulating contact was moved from medial to lateral. Adjacent contacts caused a similar movement pattern, and the transition from one mode to the next was smooth. Stimulation through the top medial contacts of the FINE stiffened the tongue longitudinally and the base of the tongue was depressed medially (Fig. 17A, arrow 1). An opening at the tongue's base was obtained in these cases, and the opening was translated into both the left and the right side oropharyngeal dilation (Fig. 17B, top medial). Stimulation through the top lateral contacts of the FINE caused the depression of the tongue muscles toward the floor of the mouth, laterally (Fig. 17A, arrow 2). There was a little change of pharyngeal area at the tongue's root. However, this translated into a dilation on the stimulated (left) side of the oropharynx (Fig. 17B, top lateral).

The bottom medial contacts of the FINE resulted in similar activation patterns to that caused by the top medial contacts: large opening of retropalatal region with depression of the medial part of the tongue (Fig.17A, arrow 4). The pharyngeal images indicate bilateral dilation of the oropharynx (Fig. 17B, bottom medial).

The bottom lateral contacts generated similar movement patterns to those contacts on the top lateral: depression on the lateral side without opening at the tongue base (Fig. 17A, arrow 3). Some oropharyngeal dilation was observed only on the stimulated (left) side (Fig. 17B, bottom lateral).

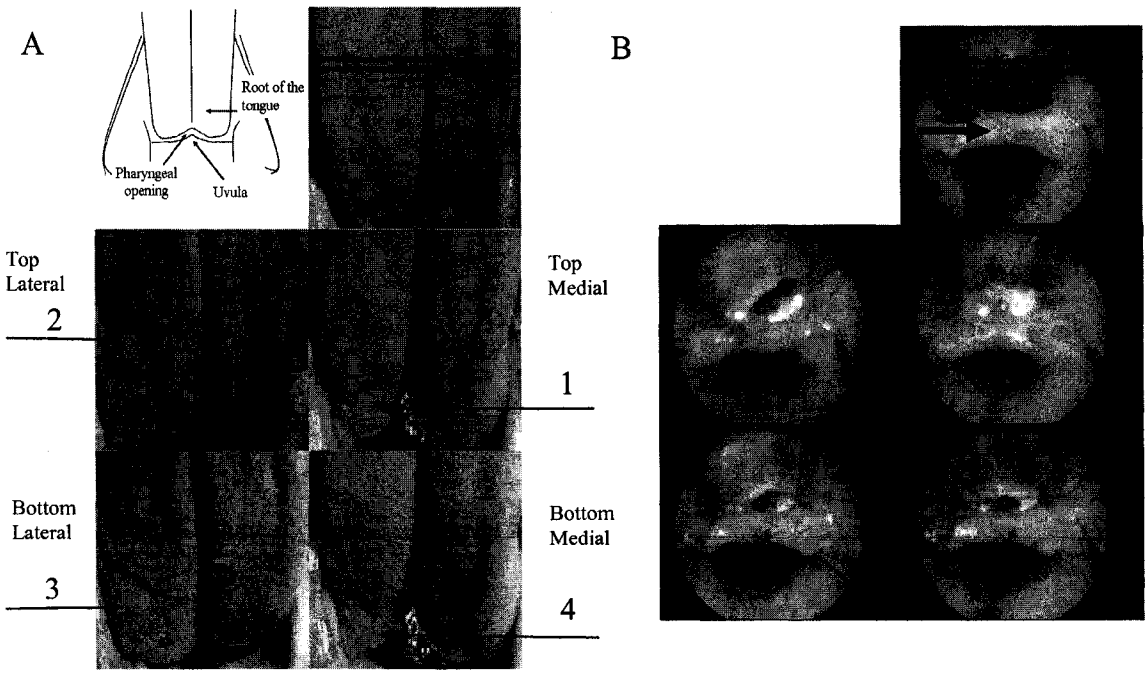

Figure 17. Tongue and pharyngeal images comparing the effects of medial and lateral contacts in an experiment. The head was 30° from the horizontal with the mouth open. Panel A: Top left drawing shows the important structures in the images. Top right: the control image at rest; the middle row: stimulation through top lateral and medial contacts of the FINE, respectively; the bottom row: stimulation through bottom lateral and medial contacts of FINE. Panel B: Corresponding pharyngeal images to the tongue images shown in A. The septum in the middle is the caudal end of the soft palate (the arrow). The nasopharynx is the opening below the soft palate and the oropharynx is the one above (dog was in supine position). The right side in the images is the stimulated (left) side of the animal.

3.3 Relationship Between Oropharyngeal Opening and Contacts of FINE

The percent of dilation in the oropharyngeal size by stimulation through each one of the 16 contacts at 30 degrees of the open mouth position in an experiment is plotted (Fig. 18A). The current level was 10% above threshold for each contact. In Fig.18B, the histology of the reshaped hypoglossal nerve from the same experiment and the estimated locations of the electrode contacts are illustrated. Stimulation through contacts 1, 3, 4, and 14 generated the largest oropharyngeal opening. Stimulation through contact 4 caused the maximum opening among all the contacts. Stimulation through contacts 2, 5

through 13, 15, and 16 caused openings less than 65% of the maximal value. The comparison of Fig. 18 A and B supports the idea that the contacts closest to fascicle 2 resulted in the largest oropharyngeal openings. Because the contact position varied with respect to the fascicle locations in each implant, the same contacts did not generate similar results in all the experiments, hence preventing us from relying on individual contacts from all experiments. However, a similar relation between the contact positions and the fascicles could be drawn in other experiments.

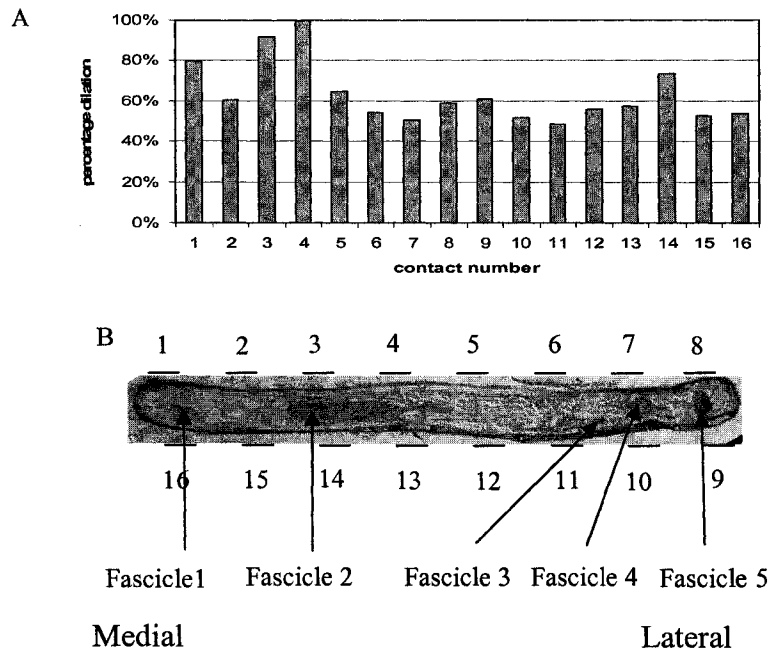

Figure 18. Oropharyngeal dilation as a function of the position of the stimulating contact around the nerve. A: The percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at 30 degrees open mouth position in an experiment. B: The histology section of the reshaped hypoglossal nerve from the same experiment and the arrangement of the FINE contacts.

3.4 Tongue Movements and Pharyngeal Opening During Branch Stimulation

There were several different tongue movement patterns observed during the branch stimulation at 10% above the threshold current level. Fig. 19 shows the different

activation patterns during the branch stimulation at 30 degrees with an open mouth position in an experiment: slight to significant closure of the retropalatal region (branch 1 and 2); mild to significant opening of the retroplatal region (branch 3 and 4); and changing of the tongue shape without opening of the retroplatal region (branch 5). Those patterns were similar to some of those obtained during contact stimulation but not exactly the same. Among those 5 branches simulated, branch 3 caused the most significant opening of the oropharynx at different head and mouth positions. Stimulation through branch 3 could open the oropharynx bilaterally (Fig 19B, middle right). Branch 4 was the second best branch. However, it could only cause a unilateral opening of the oropharynx (Fig 19B, bottom left). The other 3 branches did not open the pharynx significantly at less than 10% above threshold level, but when the current level increased to above 10%, the only pattern observed was the retropalatal opening.

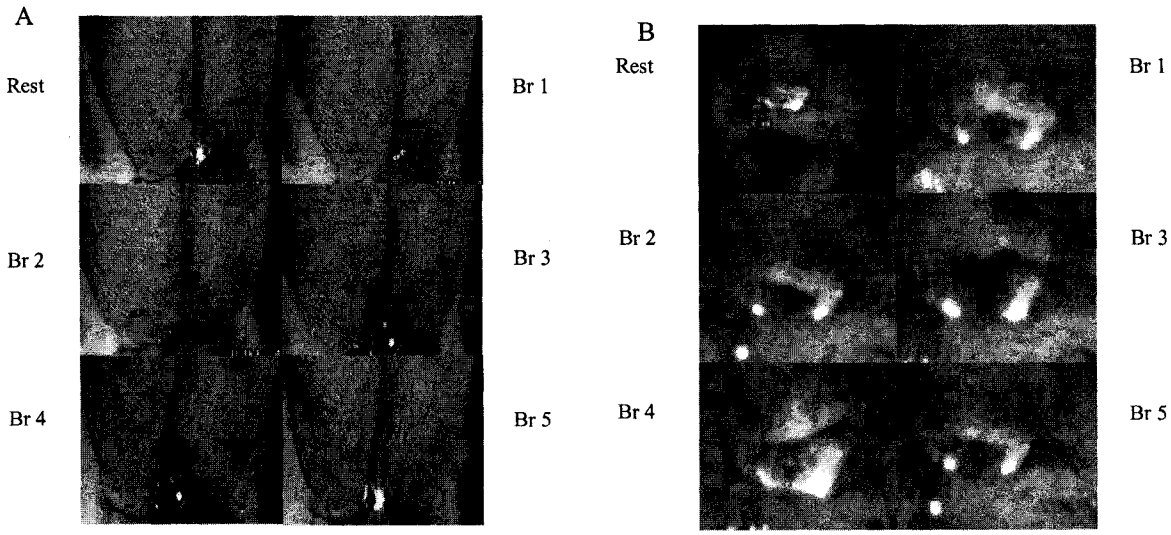

Figure 19. A. Typical images of branch stimulation taken in 30 degrees open mouth position from one experiment. B. The corresponding pharyngeal images.

3.5 Comparison of FINE Stimulation and Branch Stimulation

The branch and the FINE contact that generated the maximal oropharyngeal openings were determined using the percent dilation (Equation 1) in each experiment and various cases were statistically compared with a *t*-test. The best contact from each experiment is listed in Table 1, which was always one of the medial contacts, either on the top or the bottom of the electrode. The average percent of dilations obtained with the FINE were compared between the open mouth at 30° and 60° positions; the difference between these 2 positions is not statistically significant (α=0.1). For both of the open mouth positions, branch 3 was the most effective branch in terms of oropharyngeal opening in 8 cases out of 10. The best contact was compared with the best branch using Equation 2. As for oropharyngeal opening, at 10% above the threshold current level, there was no statistical difference between the branch and the contact groups, although for each experiment the best branch or contact could be different (α=0.1).

Table 1. Comparison of FINE and branch stimulation in terms of oropharyngeal opening. (Normalized with the best branch.)

Experiment	Open mouth 30°		Open mouth 60°	
	Best FINE contact	Contact vs. Branch	Best FINE contac	Contact vs. Branch
1	14	106	12	168
2	4	121	11	99
3	15	89	11	123
4	12	122	4	107
5	10	104	3	68
Mean ± STD		108± 12%		113± 33%

3.6 Closed Mouth Positions

In 5 out of 7 experiments, there was no oropharyngeal opening in the closed mouth positions. In those cases where there was some opening (4 cases in total), the oropharyngeal opening was measured with the same method mentioned above (Equation 1). The results were similar to those of the open mouth cases. Branch 3 was the best branch in all 4 cases (2 closed mouth 30° cases, 2 closed mouth 60° cases). The best contacts were either top (2 out of 4 cases) or bottom (2 out of 4 cases) medial contacts, which could cause dilation comparable to branch stimulation.

For the closed mouth 30° case, the percent opening with the best contact was 113 ± 34%. The same was 132 ± 36% for the closed mouth 60° case.

3.7 Medial vs. Lateral Contacts

Figure 20 through 23 show plots of the percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at different head and mouth positions.

In 5 out of 7 experiments, for open mouth 30° cases, the contacts were divided into medial (contacts 1 through 4, 13 through 16) and lateral (contacts 5 through 12) halves; there was no statistical difference between the two groups ($p=0.18$). For the open mouth 60° cases, results were similar to those from the 30° cases ($p=0.22$). That is, although the best contacts were from the top or the bottom medial contacts, overall medial contacts did not result in larger areas than the lateral contacts as a group (see Table 2).

Table 2. Comparison of medial and lateral contacts of FINE in terms of oropharyngeal opening. Normalized with the best contact.

Experiment	Open mouth 30°			Open mouth
	Best FINE contact	Contact vs. Branch	Best FINE contact	Contact vs. Branch
1	14	106	12	168
2	4	121	11	99
3	15	89	11	123
4	12	122	4	107
5	10	104	3	68
	Mean ± STD	108± 12%		113± 33%

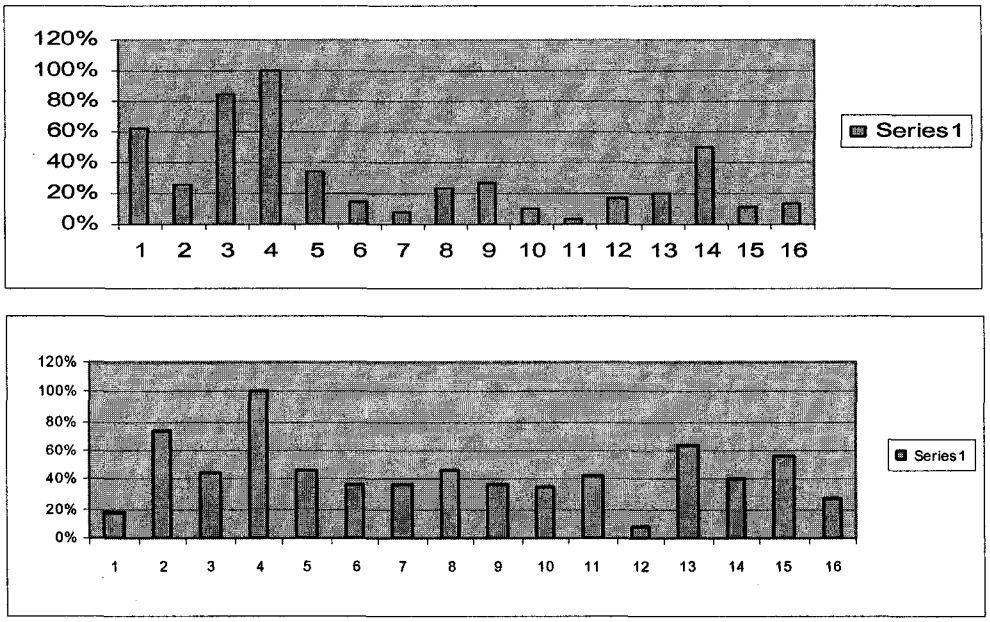

Figure 20. The percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at 30 degrees open mouth position in two experiments.

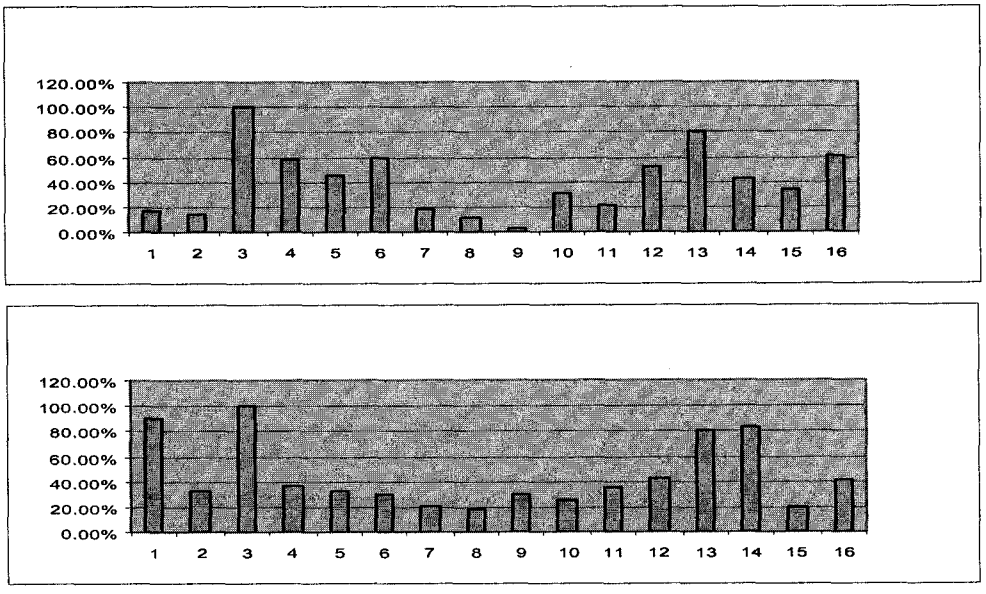

Figure 21. The percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at 60 degrees open mouth position in two experiments.

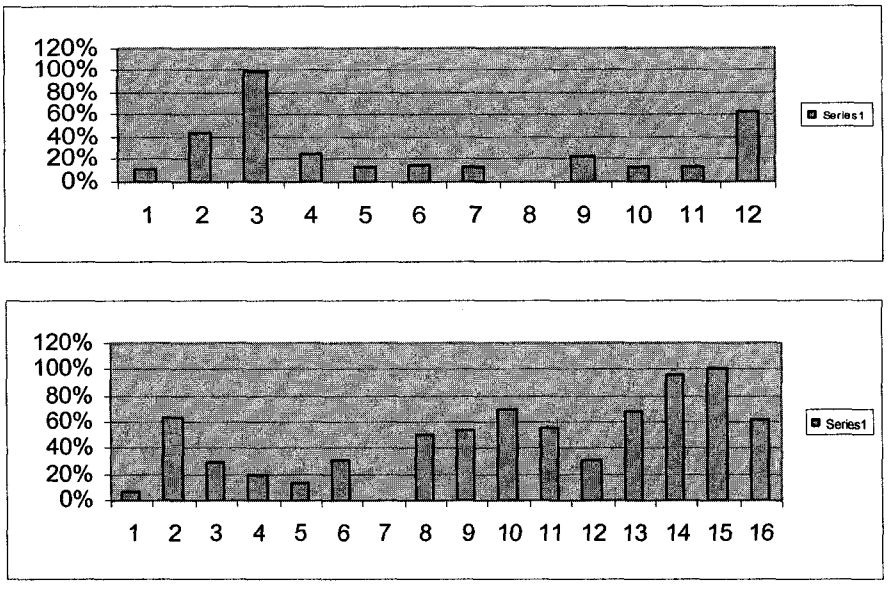

Figure 22. The percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at 30 degrees closed mouth position in two experiments.

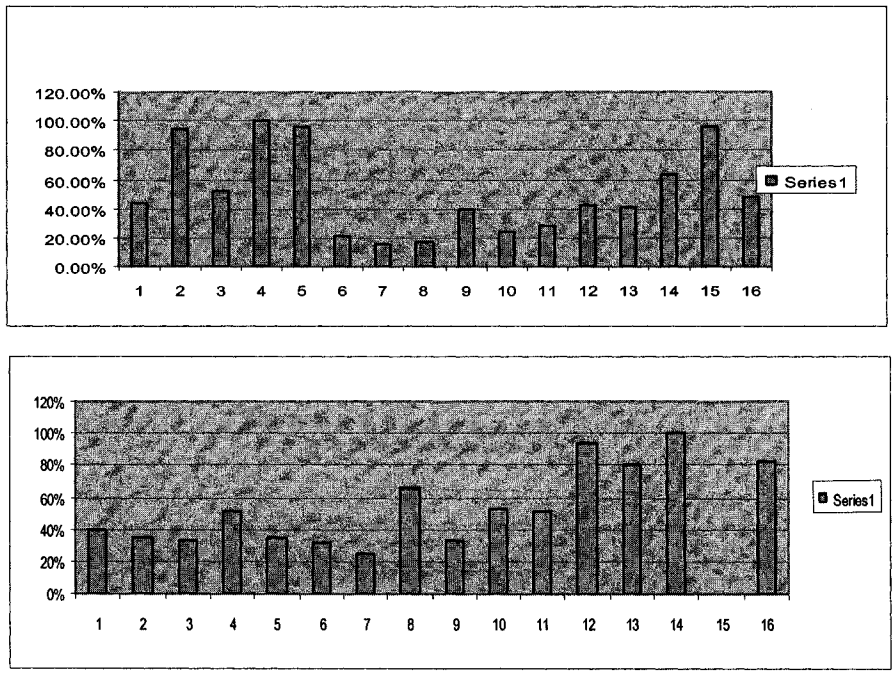

Figure 23. The percent dilation of oropharyngeal opening by stimulation through each of the 16 contacts of the FINE at 60 degrees closed mouth position in two experiments.

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Recruitment Curve

Efferent nerve supply of the muscle contains a distribution of fiber diameters [85]. Therefore, the group of nerve fibers that will be activated with an electrical stimulation is always a major concern in choosing stimulus parameters.

To evaluate nerve simulation selectivity, a recruitment curve was introduced (Fig. 20). This curve identifies the relationship between the stimulus current and the effect of stimulation (e.g. normalized force).

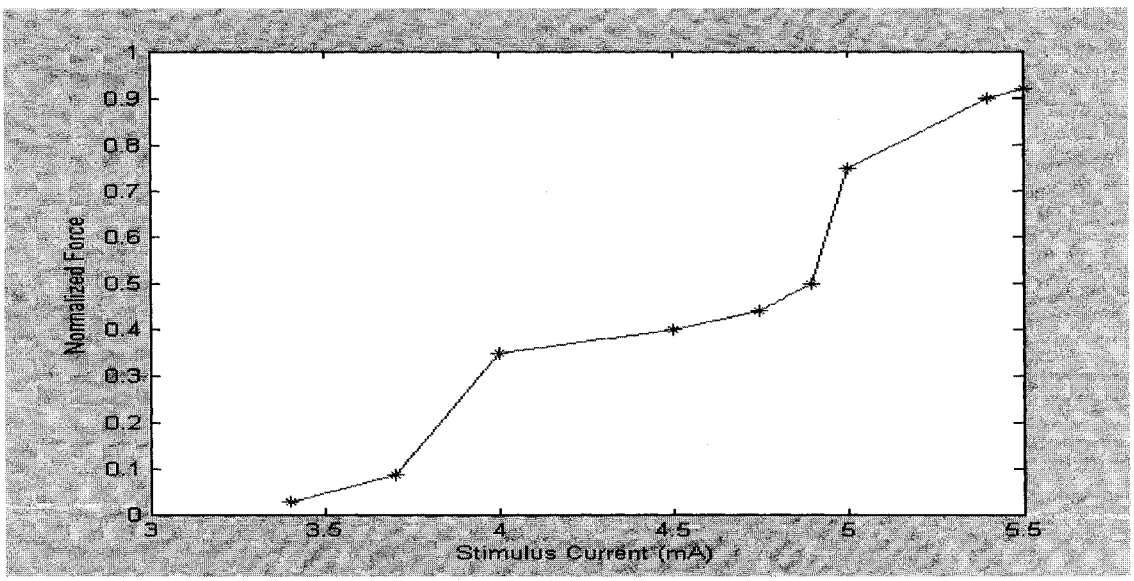

Figure 24. Activation Curve (not real data).

When the curve shifts to the right, it indicates that the threshold is higher for stimulation. The steeper the curve is, the lower the selectivity will be, i.e. in a small range of current levels, many muscle fibers are activated. With this curve, selectivity of stimulation can be quantified.

4.2 Stimulus Parameters

A wide variety of stimulation parameters can be used in nerve stimulation studies. This section describes some of the more important parameters and clarifies which ones were used in these experiments. These parameters are divided into those related to the stimulation waveform and those related to the FINE electrode configuration.

4.2.1. Stimulus Waveforms

4.2.1.1 Biphasic Waveform Biphasic (anodic-cathodic) stimulus has several advantages over monophasic cathodic stimulus [86, 87]:

1. The addition of a secondary anodic pulse balances the charge of the stimulating cathodic pulse to avoid tissue damage.

2. The addition of the secondary anodic pulse can help recruit more nerve fibers within the nerve to produce more force.

3. Biphasic stimulation can enhance the stimulation threshold difference between the nerve fibers, which means at a certain current level above threshold, biphasic stimulation would recruit a narrower range of fibers than monophasic stimulation.

In this study, only spatial selectivity is a concern. The first 2 advantages are what we need, but not the last. We are interested in activating fascicle adjacent to a specific contact before the ones away from it. The efferent nerve supply of muscle contains a distribution of fiber diameter [85], so in our case, if the distance between the fibers and

the contacts were the same, the application of biphasic waveforms activated large nerve fibers first; but when the current level increased, small fibers were activated. Pharyngeal images taken during stimulation showed that with the incensement of the current level, the pharyngeal opening increased, which means more nerve fibers were activated. The increase of the opening was not caused by recruiting nerve fibers in other fascicles because the opening pattern did not change. Also, the charge balanced stimulus reduced toxic effects.

4.2.1.2 Delay Increasing delay can cause the recruitment curve to be shifted towards lower stimulation amplitudes; that is, at a low current level, there is selectivity or the threshold for stimulation decreases [88]. When the delay between the two phased of stimulus was greater than 80 μs, little difference existed in the recruitment curves for monophasic and biphasic stimuli. In this study, the delay was 100 μs. There was no nerve fiber selective effect observed for a delay of this duration. Also, the cathodic phase of the stimulus deactivates some fibers. With delay, the deactivation effect is reduced.

4.2.1.3 Is/Ip For biphasic stimulus, when the peak secondary (cathodic) current to primary (anodic) current ratio Is/Ip is large, the second pulse has a greater effect, and more nerve fiber selectivity can be achieved. In this study, the ratio is 0.25, which does not cause much nerve fiber selectivity; that is, nerve fibers of different diameters were stimulated at the same time.

4.2.1.4 Frequency The stimulation frequency differs from research group to research group, from 30Hz to 90Hz [62, 89, 90] to achieve fused contractions in upper airway muscles. Lower frequency is desired since for an implantable device, stimulation with a lower frequency can save energy compared with a higher frequency and therefore

increase the lifetime of the device. Also, stimulation with lower frequency can prevent muscles from fatigue. In our case, we used 50Hz which was enough to cause fused contractions.

4.2.1.5. Amplitude Goodall and her colleagues found that there were 2 peaks of compound action potential (CAP) during rabbit tibial nerve stimulation [91]. The maximum large fiber response was reached when the stimulus amplitude was three to four times the excitation threshold. The small fiber response typically began to appear after the larger fiber response had reached its maximum. When a tripole was used without a transverse current, the fibers tend to be recruited around the periphery of the nerve; overlap began when only 10% of the nerve was activated. So in our study, current levels were not over 10% above the threshold to avoid spillover.

4.2.2 FINE

4.2.2.1 Size of Contacts Modeling studies indicated that smaller electrode contacts would create a more spatially isolated excitatory field than the large contacts. In this study, the size of the cathodes was 1×0.4mm which was the smallest size possible with a hand-made procedure [92].

4.2.2.2 Window Size Grill and Mortimer [92] chose an electrode providing a cuff diameter to nerve diameter ratio of 0.8 to be implanted around the peripheral nerve at the bifurcation site. This choice of electrode size provided a snug fit between the cuff and the nerve and the long-term functional stability without physiological damage to the nerve. However, it has in general been recommended that the internal diameter of nerve cuff electrodes is 40 to 50% larger than the nerve trunk diameter in order to prevent tissue damage [93]. This guideline was developed for cylindrical electrodes whose

circumference cannot increase to accommodate the swelling. The nerve can withstand the application of small forces, as long as the electrode is able to expand or reshape to accommodate the increased nerve size [80]. Unlike the cylindrical electrodes that apply forces circumferentially and reshape the nerve into a cylinder, the FINE applies forces only on two sides of the nerve to reshape it into an elongated oval. The FINE allows swelling by changing from an oval to a circular shape to accommodate a larger volume of tissue and fluid without changing the overall electrode circumference [83]. In this study, the window size was chosen based on the report from Yoo et al. [75] and the nerve was reshaped and snugly fit the FINE.

4.2.2.3 Pressure of FINE Encapsulation and scar tissue can grow between the nerve and electrode after implantation and cause the increase in nerve diameter up to 133% of the nerves' pre-implantation diameter [79, 80]. Cuoco and Durand have reported that even if the nerve swells to 133% of its resting diameter, the nerve cuff electrodes would not generate enough pressure to occlude blood flow [79]. Studies show a reduction in action potential amplitude by 70% after 270 min of compression with 30 mmHg [94]. In this study, the same contact could still produce the same oropharyngeal opening after 420 min, although the threshold for electrical stimulation increased.

4.2.2.4 Spacing of Contacts Simulation studies suggested that closer spacing between anodes and cathodes would improve spatial selectivity [92]. The fiber diameters affected by the electrode array are directly proportional to the intercathodic distance. Selective activation of small axons can be produced by an electrode array with intercathodic space equal or close to the internodal space of large axons [95, 96]. The

effect of the electrode array on the recruiting profile is independent of the stimulus pulse's width. In this study, the spacing of contacts was 1mm.

4.2.2.5 Impedances of FINE Contacts Minimizing contact impedances is desired for a neuroprosthesis to reduce discomfort. Before each animal experiment, the impedances of the contacts were tested (see Appendix E) and found to be less than 15KΩ. High impedance may be an indicator of disconnection of the contact with the wire and that problem must be fixed before animal experiments can be conducted.

Table 3. Electrode impedance results from one experiment

Electrode No: 1(The top part of FINE)

	f = 1 kHz		f = 10 kHz	
Contact No	Voltage (mV)	Resis. (kOhm)	Voltage (mV)	Resis. (kOhm)
Anode A1	88.7	1.3110	110.2	0.6280
Anode A2	79.6	1.7799	102.3	0.8968
Cathode 1	67.9	2.3835	98.0	0.9788
Cathode 2	60.8	2.9176	88.6	1.3150
Cathode 3	63.2	2.7236	88.8	1.3071
Cathode 4	57.1	3.2486	83.1	1.5470
Cathode 5	63.8	2.6774	91.0	1.2225
Cathode 6	53.8	3.5822	86.8	1.3877
Cathode 7	54.3	3.5290	86.8	1.3877
Cathode 8	55.7	3.3853	85.9	1.4252

Electrode No: 2 (The bottom part of FINE)

	f = 1 kHz		f = 10 kHz	
Contact No	Voltage (mV)	Resis. (kOhm)	Voltage (mV)	Resis. (kOhm)
Anode B1	123.9	0.3569	131.7	0.2055
Anode B2	121.6	0.4052	122.6	0.3840
Cathode 9	69.1	2.3041	95.9	1.0482
Cathode 10	63.7	2.6851	94.7	1.0892
Cathode 11	68.5	2.3435	96.0	1.0448
Cathode 12	75.0	1.9506	93.3	1.1384
Cathode 13	76.2	1.8854	103.6	0.8075
Cathode 14	78.3	1.7761	104.7	0.7760
Cathode 15	65.7	2.5367	95.3	1.0686
Cathode 16	63.1	2.7314	95.2	1.0720

Rs= 2.2 Kohm
P-P= 144.0 mV

4.2.2.6 Threshold In most cases, the threshold for one contact at different head and mouth positions were comparable. The contacts at the margin of the FINE may have a higher threshold because the contacts did not touch the nerve.

Table 4. Thresholds of electrical stimulation through each FINE contact for different head and mouth positions.

	30°		60°	
Contact No	Open Mouth (μA)	Closed Mouth (μA)	Open Mouth (μA)	Closed Mouth (μA)
Cathode 1	260	320	380	300
Cathode 2	260	260	340	260
Cathode 3	160	180	200	160
Cathode 4	180	200	180	200
Cathode 5	260	280	220	300
Cathode 6	420	420	150	420
Cathode 7	520	500	190	240
Cathode 8	700	700	420	460
Cathode 9	520	480	240	480
Cathode 10	400	360	180	380
Cathode 11	320	300	230	260
Cathode 12	160	160	160	170
Cathode 13	200	160	200	180
Cathode 14	220	220	240	200
Cathode 15	300	300	340	300
Cathode 16	360	360	400	360

4.2.2.7 Movement of FINE Grill and Mortimer reported that tissue encapsulation stabilized the electrode position and reduces significantly the degree of position dependent recruitment [77, 92]. In our study, encapsulation and the growth of scar tissue occurred right after the FINE implantation. There was no visible movement of the FINE during the experiment. It was very difficult to remove the FINE after each experiment showing that there was little chance for the FINE to move along the nerve. During each experiment, the same tongue movement mode was observed for one single contact with

the same head and mouth position, although the threshold may change. So in this study, movement of the FINE did not affect the result and conclusion.

Histology examination of the nerve tissue showed accumulation of leukocytes and active fibroblasts in the reshaped region. The possible reason for this inflammatory reaction is the reshaping and movement of the FINE relative to the underlying tissue.

4.2.2.8 Safety of FINE The stimulation thresholds of the FINE are similar to those reported for other peripheral electrode designs, such as cylinder electrodes and spiral electrodes. Also, the FINE did not reduce the number of axons conducting action potentials to the muscles. Stimulation from the FINE produced a maximum output with the same magnitude as stimulation of the individual branches distally. This result indicates that all fibers of a particular branch are excited by the FINE and conduct action potentials. Therefore, the FINE does not appear to cause acute changes in the nerve physiology [83].

4.2.2.9 Chronic Experiments In chronic experiments up to one hundred and thirty-nine days postimplantation [97], all cuff electrodes were capable of selectively activating nerve branches as well as groups of fibers within branches. There has been no histology results so far for chronic FINE implantation. Histology examination of chronic experiments using cuff electrode revealed focal areas of abnormal morphology including perineural thickening, proliferation of endoneural connective tissue, and thinned myelin [92]. These morphological changes could have resulted in variations of stimulation results in chronic cases.

4.3 Imaging During Stimulation

The complex but precise movements of the tongue depend on the coordinated actions of its extrinsic and intrinsic muscles. The extrinsic muscles are the styloglossus (SG), hyoglossus (HyG), and genioglossus (GG). The function of the GG is to depress and protrude the tongue, while the function of the styloglossus and the hyoglossus is to retract the tongue. The function of the geniohyoid (GH) is to draw the hyoid apparatus cranially and to maintain a patent airway. All of the above muscles are responsible for upper airway patency and innervated by the HG nerve [3]. In this study, the activation patterns of the tongue were studied during selective stimulation of the HG nerve with a multi-contact electrode implanted on the nerve trunk and cuff electrodes implanted on the branches. Activation patterns of the tongue and the corresponding pharyngeal changes were observed. There have been a number of studies on the functional outcome of the HG nerve stimulation using muscle activity, air flow, or critical closing pressure. This study makes an attempt to obtain more functional information by employing the imaging method while activating the tongue muscles, selectively.

Only the oropharyngeal area was calculated since no nasopharyngeal opening was observed in any of the experiments. The middle and caudal portions of the nasopharynx are attached dorsally to the base of the skull and the muscles such as the pterygopharyngeus and the palatopharyngeus constrict and draw the nasopharynx forward. The ventral boundary of the nasopharynx is the mobile soft palate. The muscles of the soft palate consist of the paired palatine muscles, tensor and levator veli palatine muscles. None of the muscles responsible for reshaping the nasopharynx is innervated by the HG nerve, so it is not surprising that during the HG nerve stimulation, the

nasopharyngeal area did not change [3]. The change in the oropharyngeal area also must be due to passive traction of the muscles since the HG nerve does not control any pharyngeal muscles. This also explains why the upper airways were sometimes closed at the base of the tongue, as observed in the transoral images, without a change in the oropharyngeal caliber. In this study, the tongue retraction was not observed in open mouth positions because the tongue was pulled out and hung from the tip using a rubber band.

It was visually evident that simulation of the HG nerve changed airway size and configuration both within and outside the cross-sectional plane selected for measurements. It was also evident that nerve stimulation caused structures to move in both radial and axial direction. The axial movements in this study were much less significant compared with the study by Kuna [4] because the tip of the tongue was mechanically stabilized. Although the axial movements could not be controlled or quantified, it is felt that they did not significantly affect cross-sectional measurements. The distance between the tip of the fiberscope and the rim of the soft palate was measured and it did not change with nerve stimulation.

Our data agreed with results of other studies that significant changes in airway caliber with nerve stimulation were associated with significant unidirectional changes in both maximum anteroposterior and lateral diameters. FINE stimulation produced similar changes in anteroposterior and lateral dimensions at the given airway level. These results indicate that the changes in the airway area in response to contraction of the tongue muscles were concentric [4]. Airway dilation of the hypoglossal nerve appear to involve complex mechanical relationships, whereby ventral displacement and ventral-lateral

stretch of the pharyngeal wall tissues causes significant increases in both the anterioposterior and the lateral airway dimension [98].

Kuna [4] reported that activation of the tongue muscles that lie on the ventral surface of the oropharynx can dilate significantly the velopharynx. This may be due to the tethering effects of tongue movement pulling on soft tissue structures attached to the tongue and soft palate. Alternatively, tongue movement may have altered the position of the hyoid bone, causing a secondary enlargement of the pharynx via other fibromuscular attachments to the hyoid.

Results from this study suggest that passive traction of the muscles dilates the oropharynx when the HG nerve is stimulated in the dog. Whether the activation of the tongue muscles passively causes dilation in the nasopharynx depends on the anatomy. In the dog, this did not occur for the positions of the head and the lower jaw studied. However, nasopharynx dilation may be a possibility in the human subjects. In OSA patients, the occlusions happen at the oro- or nasopharynx. Thus, dilation of either one of the pharyngeal openings may be needed depending on the individual case.

In the current study, electrical stimulation through the medial contacts of FINE caused bilateral oropharyngeal opening while the lateral contacts caused only an opening on the stimulated (left) side. A possible explanation is the fibers of the medial HG nerve branches may crossover to the contralateral muscle of the tongue [3]. The opening was both in the anteroposterior and the mediolateral direction for medial contact stimulation. For branch stimulation, only branch 3 caused bilateral oropharyngeal opening; all other branches only caused an opening on the stimulated side.

The FINE works on the principle that a peripheral nerve can be reshaped slowly without introducing trauma [82]. The window size of the electrode (8mm by 0.6mm) was chosen to reshape the HG nerve by aligning the fascicles without significantly reshaping them [83]. Histology results presented and the mere observation of the explanted nerves indicated that the HG nerve was reshaped to the resting window size of the electrode. The fascicles in the middle of the nerve were closer to the contacts after reshaping. Therefore, the current needed to active a fascicle should be reduced after reshaping. More importantly, the fascicles underneath the contacts can be activated without activating other fascicles, i.e. spatial selectivity can be achieved. In this study, at large current levels (usually > 2 times the threshold), the tongue activation pattern was the same regardless of the contact used; this indicates that the current was strong enough to activate the whole nerve. Thus the current was limited at 10 to 20% above the threshold to achieve functional selectivity. Small stimulation currents may also be preferable in the clinical application in order to minimize disturbance to the patient.

Our data agreed with the results of Yoo et al. [83], that medial contacts stimulated fascicles of branch 1, 2, and 3 to cause the activation of GH and GG; the lateral ones stimulated fascicles of branch 4 and 5 to activate HyG and SG. Contacts 3, 4, and 14 are adjacent to fascicle 2 and at activation mode caused by electrical stimulation through those contacts were similar to that caused by branch 3 stimulation. So it is possible that fascicle 2 became branch 3 beyond bifurcation site. However, a detailed axonal tracing study was not conducted to map the separation of fascicles into the 5 major branches observed. There were some small nerve fibers proximal to the implantation site and away from the bifurcation site which were not contained by the stimulating electrode.

Two different head (30° and 60°) and mouth positions (open and closed) were used to assess the function of HG nerve stimulation. Oroharyngeal openings at the 30° position were larger than the 60° position, which agrees with former studies [44]; however, the difference is not statistically significant. For all four cases (head 30° or 60° from horizontal plane with the mouth open or closed), the results were similar. In open mouth cases where transoral images with a camcorder were available, medial contacts caused medial depression of the tongue base while lateral contacts caused lateral depression of the tongue base.

Spontaneous breathing was suppressed by mechanically ventilating the animal. No significant change of oropharyngeal opening was observed during inspiration or expiration. Thus, synchronization of the stimulations with breathing was not needed to eliminate the effect of respiration in the images.

Anesthesia reduces upper airway muscle activity compared to awakened state thereby affecting the airway measurements [99]. Moreover, the anesthesia could cause partial or complete airway obstruction during data acquisition [100]. To overcome these side effects and reduce edema, dexamethasone was administered, resulting in a slightly open oropharynx at the resting state.

Optical deformation introduced by lenses of endoscope makes it difficult to determine the pharyngeal opening quantitatively, especially when large angular field lenses are used. Moreover, when the plane of the object of interest is not parallel to the plane of lenses, the area measurements may not be accurate. Lafortuna and his colleagues implemented an algorithm for the correction of the deformation [101]. We did not calculate the actual areas in this study; instead, numbers of pixels in the areas of

interest were counted and used as a relative measure of area. Because the distance and the angle between the plane of the lenses and of the target structures did not change during the experiments, the deformation factor was not accounted for in calculation of the percent opening.

4.4 Histology

Thin (7μm) slides show the accumulation of neutrophils and fibroblasts between fascicles indicating acute inflammatory reaction of the nerve tissue to the reshaping. Thick (20μm) slides provide the configuration of the fascicles showing a consistent fascicular organization at the site of electrode implantation. The nerve image in Fig. 21 provides a good example: larger fascicle (fascicle 2) is centered between fascicle 1 and other small fascicles.

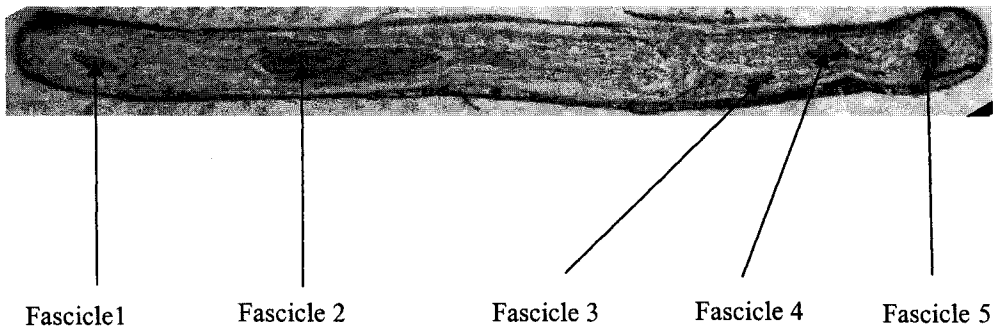

Figure 25. The histology of the reshaped HG nerve with 5 major fascicles.

4.5 Conclusion

Electrical stimulation of the HG nerve through the FINE contacts provides various tongue activation patterns, some of which can translate into oropharyngeal openings. These results suggest that selective stimulation of the HG nerve can be a useful technique to maximize the effects of HG nerve stimulation in removing the obstructions in sleep apnea patients. Having multiple activation patterns for dilating the UAW can be important for two reasons. First, this may increase the size of the patient population who can benefit from HG nerve stimulation since the type of occlusion may be different in each patient. Second, muscle fatigue that may result from long hours of electrical stimulation can be prevented by switching between different stimulation patterns.

4.6 Future Directions

The current study is preliminary. Research of the next stage includes:

- Comparation of longitudinal stimulation with the transverse stimulation; stimulation through combined contacts instead of only one contact. The current research shows single contact stimulation is as good as branch stimulation, but not significantly better. Combined contact stimulation may be better than branch stimulation.
- Chronic animal experiments are needed to verify the safety and effectiveness of FINE. The effect of scar tissue growth, long term safety and efficiency of the HG nerve stimulation with the FINE need to be tested. In this case, synchronization of stimulation with animal's spontaneous breathing must be taken into consideration.
- Research on human subjects is needed to verify the conclusions drawn from animal experiments.

APPENDIX A:

FABRICATION OF FINE

Hand-made FINEs were used in the first 3 experiments. The basis of fabricating the multi-contact nerve cuff electrodes is curing elastomer within a cusom-made mold (Fig. 21).

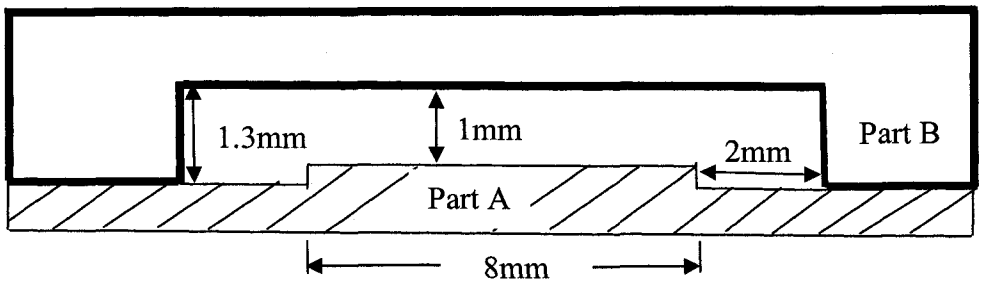

Figure 26. The mold for making FINE.

The stainless-steel mold consisted of two pieces. Elastomer was filled in the gap between them with contacts sitting on Part A. The process will be described in detail.

2.1 Electrode Contacts

Eight pieces of platinum (4mm×0.5mm, 25μm thickness) were cut and spot-welded with insulated stainless-steel wires (316LVM, 7-strand, teflon coated, diameter 0.18 mm without insulation, 0.55 mm with insulation, Fort Wayne Metals) and used as big contacts. Another eight pieces of platinum (0.5×0.5mm, 25μm thickness) were cut and spot-welded with insulated stainless wire and used as small contacts.

The big contacts were glued on a single sheet of silicone (50 μm thick) with epoxy as an array with inter-contact distance of 1.1 mm. The wires exit the array in the same direction. A small amount of silicone elastomer (Dow-Corning MED4-4210) was degassed within a vacuum desiccator and applied over the array.

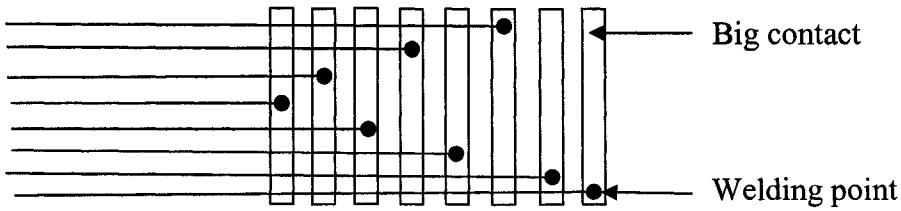

Figure 27. Big contacts with wires.

After curing, this silicone piece was turned over. Each small contact was glued at the middle site of each big contact on the other side of the silicone piece.

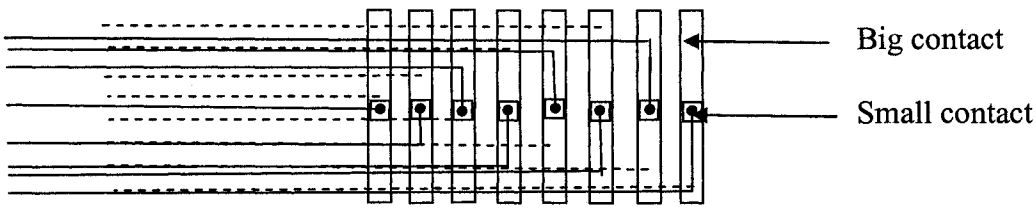

Figure 28. Small and big contacts put together.

Part A of the mold was sprayed with Mold Releases/Lubricants (LPS Dry Film, LPS Laboratories, Tucker, GA). Then the silicone piece with large and small contacts was put on the ridge of Part A with small contacts facing it. The gap between the two parts of the mold was then filled with silicone elastomer and the two parts were put together tightly. Following complete cure, excess elastomer was cut away. Windows of 0.25×0.25mm were made with a scalpel on the cured silicone elastomer to expose the small contacts; the exposed areas would be the cathodes. Also, windows of the same size were made at each end of each big contact; those exposed areas would be anodes.

Using the same method, another eight-contact array was made.

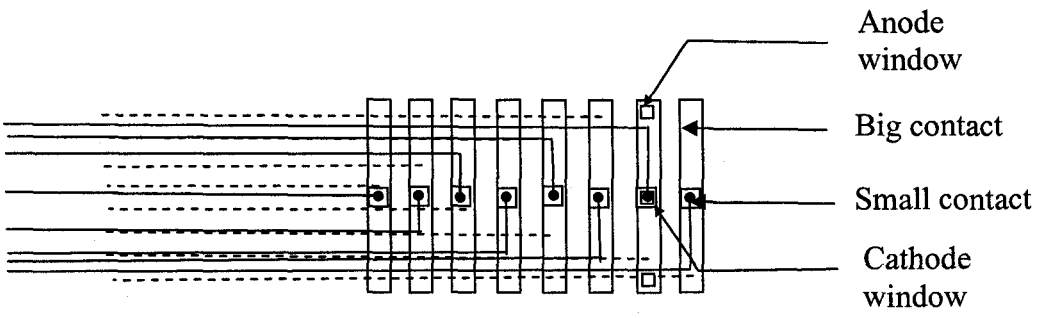

Figure 29. Making windows on the contacts.

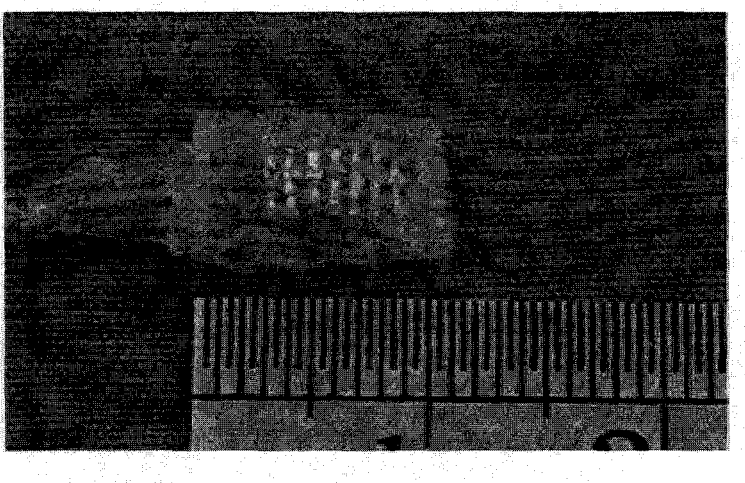

Figure 30. Contacts of FINE mounted on the silicone substrate.

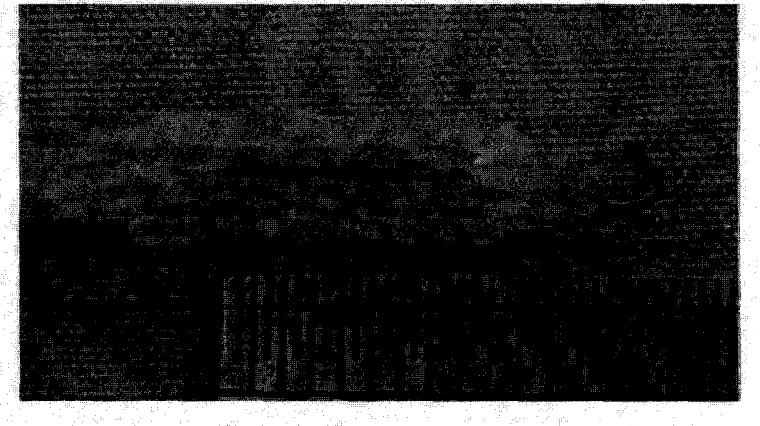

Figure 31. The cross-section of FINE showing the window.

The two tripolar arrays were then bonded facing each other with silicone elastomer. The contacts were symmetrically located within the inner side walls of the FINE.

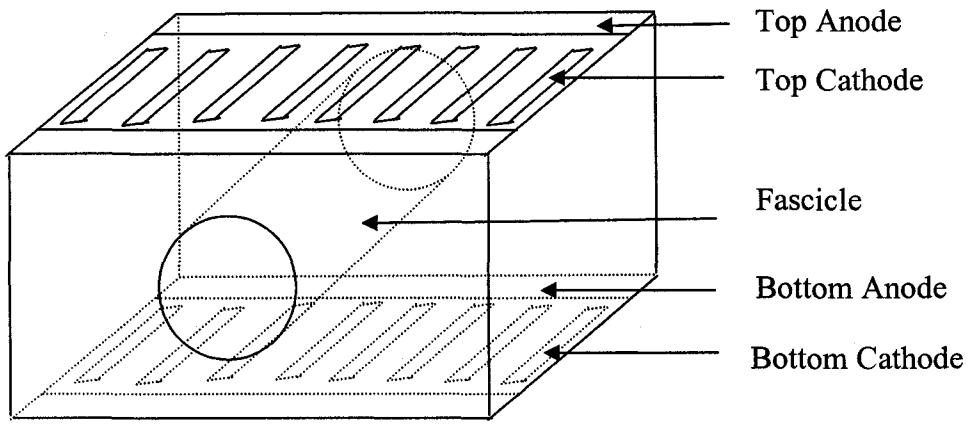

Figure 32. Fascicle going through FINE.

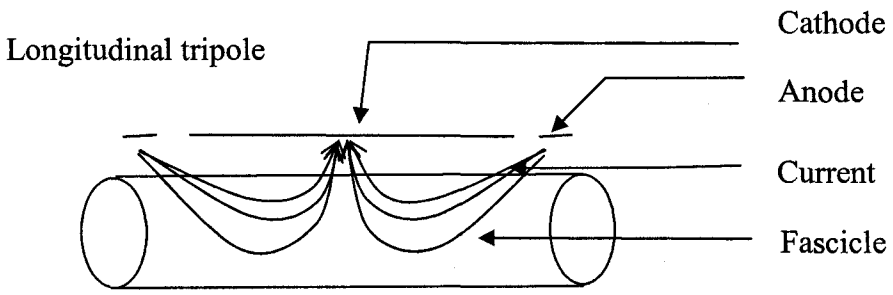

Figure 33. Cathodic activation.

APPENDIX B:

TISSUE PROCESSING

The reshaped section of the HG nerve was explanted, removed and fixed in 4% buffered paraformaldehyde solution for at least 48 hours. Then the nerve tissue was rinsed in running water to remove most of the fixative and put in a tissue capsule for processing. The following steps were performed sequentially for dehydration, clearing, and infiltration procedures:

1. Kept in 75% ethyl alcohol overnight (can be kept for three days).
2. Kept in 95% ethyl alcohol for a minimum of two hours.
3. Kept in 100% ethyl alcohol-1 for a minimum of two hours.
4. Kept in 100% ethyl alcohol-2 for a minimum of one hour.
5. Kept in Histoclear-1 for a minimum of two hours.
6. Kept in Histoclear-2 for a minimum of one hour.
7. Kept in 1:3 Paraffin-Histoclear mixture overnight.
8. Kept in 2:3 Paraffin-Histoclear mixture for about two hours.
9. Kept in pure paraffin for about two hours in oven.
10. Kept in pure paraffin for about two hours in vacuum oven.

The tissue capsule was taken out of the vacuum oven and kept under an infrared heating lamp to make it ready for embedding. A metal mold was taken, and an embedding ring was fixed over it. Liquid paraffin was poured into the mold, filling it up to the brim. The HG nerve tissue was dropped into the mold and oriented accordingly. The mold was cooled and the embedding ring lifted up to get a paraffin block with tissue embedded in it. Before fitting the block in the microtome, the excess of paraffin was removed. A fresh disposable microtome knife was taken and sections of the HG nerve with a thickness of 20 μm were cut and put over the water surface to spread. A small

pinch of jelly powder was spread over the water, which acted as a glue to stick the section on the slide surface. Two to four sections were loaded on each slide. The slides were labeled for staining procedure.

Hematoxylin-Eosin Staining

The following steps were performed sequentially for removing paraffin, hydration, hematoxylin staining, dehydration, and eosin counterstain procedures:

1. Kept in Histoclear I for at least 3 minutes.
2. Kept in Histoclear II for at least 3 minutes.
3. Kept in 100% Ethanol for 2 minutes.
4. Kept in 95% Ethanol for 2 minutes.
5. Kept in 70% Ethanol for 2 minutes.
6. Kept in 50% Ethanol for 2 minutes.
7. Kept in water for 2 minutes.
8. Kept in Hematoxylin working solution for at least 20 minutes.
9. Water rinse for 2 minutes.
10. Kept in $NaHCO_3$ (bicarbonate) solution for 2 minutes.
11. Kept in water for 1 minute.
12. Kept in 50% Ethanol for 2 minutes.
13 Kept in 70% Ethanol for 2 minutes.
14. Kept in Eosin in 90% Ethanol for 2 minutes.
15. Kept in 95% Ethanol for 1 minute.
16. Kept in 100% Ethanol for 1 minute.
17. Kept in Histoclear I for 2 minutes.

18. Kept in Histoclear II for at least 2 minutes.

Coverglasses were mounted over the slides and the slides were placed on a warming tray (35° C) overnight.

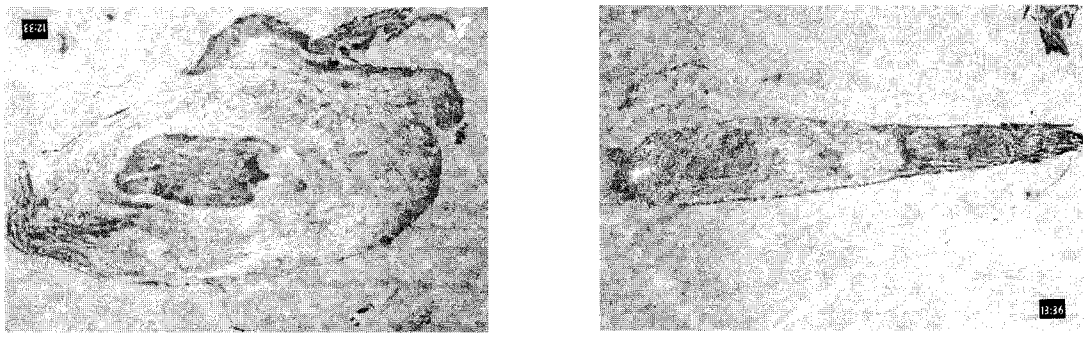

Figure 34. Comparison of HG nerve without and with reshaping.

APPENDIX C:

SWITCH BOARD

To apply electrical stimulation through each contact of FINE, a switchboard was developed. DPDT (double pole double throw) switches were used to connect the stimulus

isolator and the FINE.

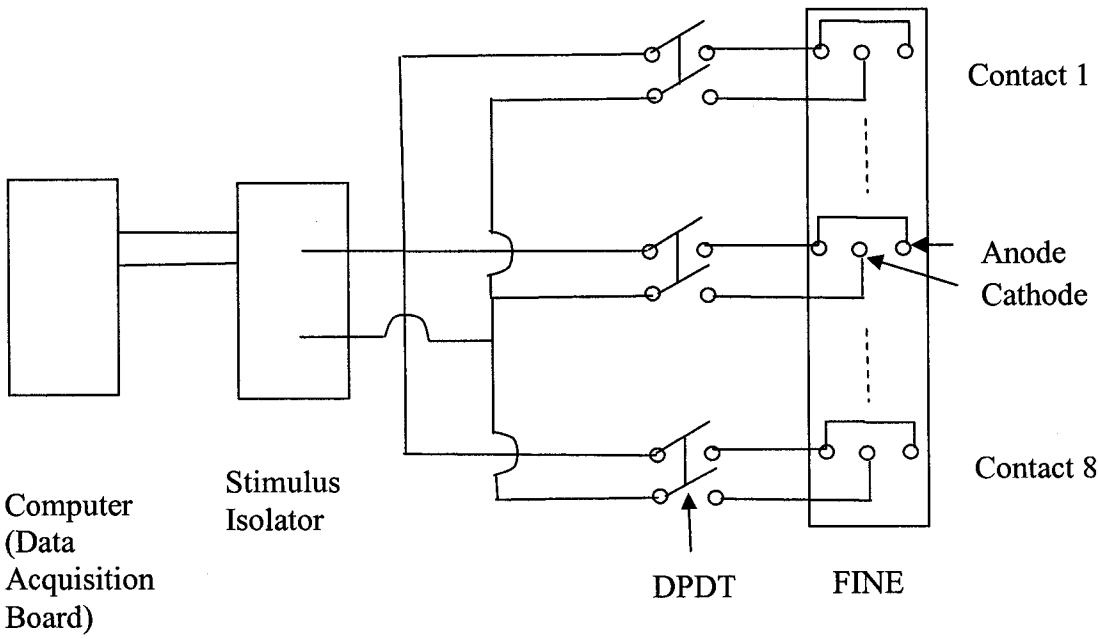

Figure 35. Switchboard.

APPENDIX D:

MATLAB CODE FOR AREA CALCULATION

```
% Calculates area of interest
% 09/14/2003 by Jingtao Huang

% A simple function to read the input image into the variable I
function c=ourarea (x)
I=imread (x,'bmp');
end
%main program
%shows the image for the user
I=rgb2gray (I);
Imshow (I);
 % allows the user to select perticular pixels surrounding the area of interest
newpic=roipoly;
 figure;
% shows the selected part of the picture in binary form
 imshow (newpic);
% calculates the area of selected region of the image
 c=bwarea (newpic)
```

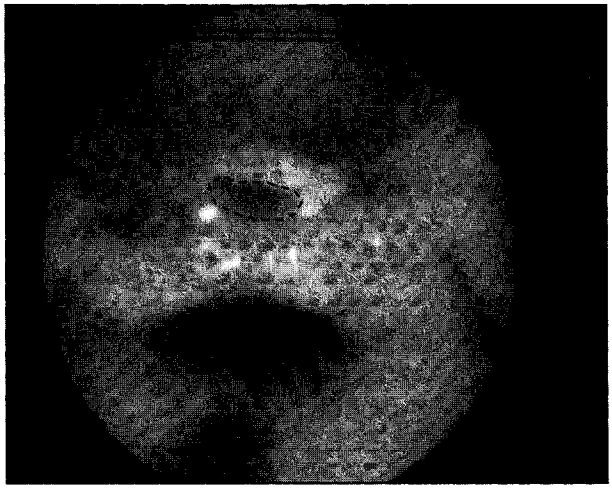

Figure 36. The pharyngeal image with the margining of the oropharyngeal opening.

APPENDIX E:

IMPEDANCE MEASUREMENTS OF THE STIMULATING ELECTRODES

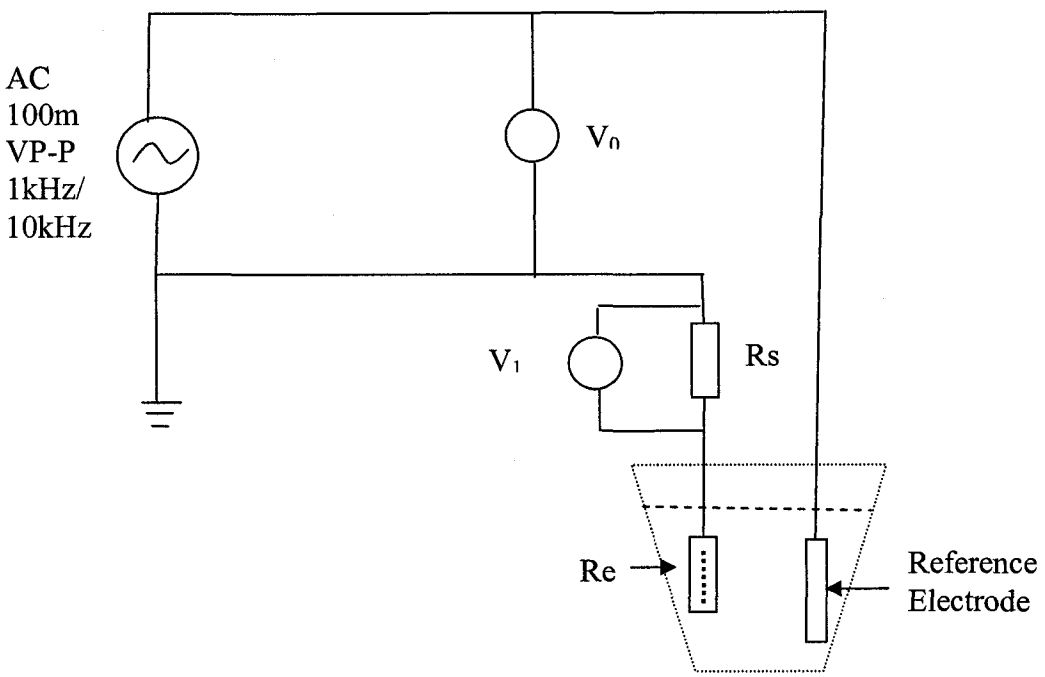

Figure 37. Setup for impedance measurement.

Each time, one contact (anode or cathode) was in the saline solution and hooked up in the above circuit. The impendance (Re) of it was calculated using the formula:

$$\mathrm{Re} = Rs \times (\frac{V_0}{V_1} - 1)$$

Rs was known to be 2.2 KΩ. The AC source provided a voltage of 144mV (P-P) with a frequency of 1 or 10k Hz sinusoidal.

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