Functional magnetic resonance imaging study of central neural system control of voice, with emphasis on phonation in women with muscle tension dysphonia

Maryna Kryshtopava

200 JAAR
UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

# FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY OF CENTRAL NEURAL SYSTEM CONTROL OF VOICE, WITH EMPHASIS ON PHONATION IN WOMEN WITH

# MUSCLE TENSION DYSPHONIA


**Maryna Kryshtopava**

**Promotor: Prof. Dr. S. Claeys**
**Copromotor: Prof. Dr. K. Van Lierde**


**2017**

FACULTY OF MEDICINE AND HEALTH SCIENCES
DEPARTMENT OF OTHORHINOLARYNGOLOGY

# FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY OF CENTRAL NEURAL SYSTEM CONTROL OF VOICE, WITH EMPHASIS ON PHONATION IN WOMEN WITH MUSCLE TENSION DYSPHONIA

MARYNA KRYSHTOPAVA

Promotor: Professor Dr. S. Claeys
Copromotor: Professor Dr. K. Van Lierde

A THESIS SUBMITTED TO FULFILL THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN HEALTH SCIENCES

2017

**Promotor**
Professor Dr. S. Claeys

**Copromotor**
Professor Dr. K. Van Lierde

**Advisory Committee**

**Examining board:**

1. Prof Dr Eric Achten, UZGent. Department of Radiology and nuclear medicine, De Pintelaan 185, K12, 9000 Gent. rik.achten@ugent.be
2. Dr. Wouter Huvenne, UZGent. Dienst Hoofd-, hals-, maxillo-faciale heelkunde, De Pintelaan 185 , 9000 Gent Wouter.huvenne@ugent.be
3. Prof D. Morsomme, Department of Logopedics. Voice Speech Unit (B38), Rue de l'Aunaie, 30 4000 - Sart Tilman 04 / 366.51.76, dominique.morsomme@ulg.ac.be
4. Prof Marc De Bodt, UZA, Dienst NKO&Hoofd-Halsheelkunde. Wilrijkstraat 10, 265. Edegem. Marc.debodt@uza.be
5. Prof Miet De Letter. Ugent, Vakgroep Spraak-taal en gehoorwetenschappen. De Pintelaan 185, 9000 Gent. Miet.deletter@ugent.be

This doctoral thesis has been funded with support Whole Europe Beyond Borders (WEBB) Erasmus Mundus Action 2 Lot 5 – Strand 1 Grant from the European Commission.

# TABLE OF CONTENTS

Dedicated to my parents and family whom i love forever

# ACKNOWLEDGEMENTS

I am greatly indebted to my promotor Professor S. Claeys and copromotor Professor K. Van Lierde for all of their help, encouragement, and support. This work would not have been be possible without them. I especially want to thank Professor H. Vermeersch for being a great friend and teacher, for the many insightful conversations I had with him over these years, and for giving me the opportunity to spend four great years at Ghent University and University Hospital. I also want to thank E. D'Haeseleer and I. Meerschman who for going the extra mile with me.

I am grateful to Professor G. Vingerhoets who, no matter how busy he was, spent time helping me with my work. I would like to thank him for his uncompromising rigor in data analysis work and for all of the functional imaging skills he taught me.

My gratitude also goes to Professor C. Bachert who allowed me to prolong my study and gave me a possibility to work at the Upper Airways Research Laboratory.

My greatest thanks go to all those who were my friends and colleagues during this exciting period of my life.

Chapter 2 is based on joint work with G. Van Maele, B. Boehme, N. Piens, K. Vonck. Chapters 3 and 4 of this thesis are based on joint work with G. Vingerhoets, E. D'Haeseleer, I. Meerschman, and P. Vandemaele. Chapter 3 is based on joint work with the medical students Michiel De Moor, Charlotte Defrancq, and Zoe Thijs.

Most importantly, I want to thank my mom, dad, brother, husband, and my children for their love and support. I dedicate this work to them.

# SUMMARY

Human phonation is a laryngeal motor behavior that extends from reflexive and unlearned limbic laryngeal actions to controlled and coordinated, highly skilled vocal movements that support speech and/or singing. Phonation requires coordination of the respiratory, laryngeal, and articulatory systems. Abnormalities or changes in phonation mechanisms result in voice disorders. When voice disorders develop in the absence of anatomical, neurological, and/or psychogenic causes, a functional voice disorder occurs. The prevalence of functional dysphonia in the treatment seeking population at our clinic is 41%, and female professional voice users are predominantly affected (43% women vs. 36% men). Most functional voice disorders fall under the category of muscle tension dysphonia (MTD) and are linked to inappropriate muscle activity in response to sensory perturbations, such as upper respiratory infections, smoke, laryngopharyngeal reflux (LPR), significant vocal demands or stressful life events. Additionally, during vagal nerve stimulation (VNS) functional dysphonia can occur (in 66% of cases), which is an example of neurological interference in laryngeal muscle activity. Study of the impact of VNS on vocal characteristics presents a unique possibility to assess voice alteration by mimicking functional voice disorders. However, central neural system (CNS) control of voice in patients with MTD, remains poorly understood. In fact, even in healthy people, the neurophysiological mechanisms of how the brain controls phonation are practically unknown.

The goal of this study was to investigate CNS control of voice with emphasis on phonation in women with MTD. The most important steps toward attaining this goal included (1) investigation of the impact of VNS on vocal characteristics, (2) investigation of CNS control of voice in healthy women and women with MTD; (3) investigation of CNS control of voice in healthy female singers with normal vocal characteristics and supraglottic compression.

The study of the impact of VNS on vocal characteristics has demonstrated that subjects with VNS had a disordered perceptual and objective vocal quality. During stimulation and especially during raised stimulation, the fundamental frequency was significantly increased. This is an example of influence of neurological perturbation on vocal output.

The study of CNS control of voice in healthy women and women with MTD has demonstrated that MTD patients use the auditory, motor, frontal, parietal, and subcortical areas for phonation control. Healthy people for the purpose of phonation control also use these brain areas. However, comparison of phonation tasks in the two groups (MTD patients and healthy individuals) revealed *higher* brain activities in the precentral gyrus, inferior, middle and superior frontal gyrus, lingual gyrus, insula, cerebellum, midbrain, and brainstem as well as *lower* brain activities in the cingulate gyrus, superior and middle temporal gyrus, and inferior parietal lobe in the MTD group. In patients with MTD, these altered (higher/lower) brain activities may result in laryngeal tension and voice symptoms.

The study of CNS control of voice in healthy female singers with supraglottic compression has showed a significant effect of phonation control in the bilateral pre/postcentral gyrus, and in the frontal, cingulate, superior and middle temporal gyrus, as well as in the parietal lobe, insula, lingual gyrus, cerebellum, thalamus, and brainstem. These activation areas are consistent with previous reports using other functional magnetic resonance imaging (fMRI) protocols. In addition, a significant effect of phonation was found in the bilateral superior temporal gyrus, and the pre/postcentral gyrus. In healthy female singers with supraglottic compression, the brain activity in the pre/postcentral gyrus may be a biomarker of laryngeal supraglottic compression during phonation.

The major challenge for future research in CNS control of voice consists of defining the connections between routine voice diagnostic behavioral measures (i.e. perceptual, acoustic, and aerodynamic) and brain imaging data in order to better understand the relationship between current clinical voice measures and the underlying neural events in disordered voice. Moreover, a study group including neurologists, otolaryngologists and speech pathologists will be needed to conduct and assess these investigations. A better understanding of CNS control of voice will help to establish biomarkers of disease and may eventually lead to individualtreatment plans that may improve clinical outcomes in the treatment seeking population of professional voice users.

# SAMENVATTING

Stemgeving bij de mens is gebaseerd op laryngaal motorisch gedrag dat varieert van reflectoire en spontane limbisch gestuurde laryngale acties tot gecontroleerde, gecoördineerde en erg gevorderde vocale bewegingen ter ondersteuning van spraak en/of de zang. Fonatie vereist coördinatie van de respiratoire, laryngale en articulatorische systemen. Afwijkende fonatiemechanismen resulteren in stemstoornissen. Stemstoornissen zonder anatomische, neurologische en/of psychogene oorzaken, worden beschouwd als functionele stemstoornissen. De prevalentie van functionele dysfonie in de consulterende populatie is 41% en het zijn vooral vrouwelijke professionele stemgebruikers die getroffen worden ( 43% vrouwen vs 36% mannen). De meeste functionele stemstoornissen vallen onder de categorie van MTD (muscle tension dysphonia). MTD wordt vooral veroorzaakt door ongepaste spieractiviteit ten gevolge van sensorische afwijkingen, zoals bij bovenste luchtweginfecties, (passief) roken, laryngofaryngeale reflux (LFR), overmatig stemgebruik of stress. Bij nervus Vagus stimulatie (NVS) kan een functionele stemstoornis uitgelokt worden. NVS kan als voorbeeld van perifere neurologisch beïnvloeding van (afwijkende) spieractiviteit in de larynx beschouw worden. De aansturing door het centrale zenuwstelsel (CZS) van de fonatie bij patiënten met functionele stemstoornissen is echter momenteel niet gekend en zelfs bij gezonde mensen is het neurofysiologische mechanisme over hoe hersenen de fonatie controleren vrijwel onbeschreven.

Het doel van deze studie was om de centraal neurologische stemcontrole te onderzoeken, daarbij vooral gefocust op fonatie bij vrouwen met MTD. De studie werd als volgt opgebouwd: (1) onderzoeken van de impact van VNS op de objectieve en subjectieve vocale karakteristieken, (2) onderzoeken van de CNS stemcontrole bij gezonde vrouwen en bij vrouwen met MTD, (3) onderzoeken van de CNS stemcontrole bij gezonde zangeressen met normale vocale karakteristieken en supraglottische compressie.

De studie naar de impact van VNS op de objectieve en subjectieve vocale karakteristieken heeft aangetoond dat personen met VNS een gestoorde perceptuele en objectieve stemkwaliteit hadden. Tijdens stimulatie en vooral tijdens verhoogde stimulatie, steeg de fundamentele frequentie significant. Toch ervaren de personen geen psychosociaal belemmerend effect op de stemkwaliteit of op de levenskwaliteit.

De studie naar CNS stemcontrole bij gezonde vrouwen en vrouwen met MTD heeft aangetoond dat patiënten met MTD de auditieve, motorische, frontale, pariëtale en subcorticale zones voor fonatie controle gebruiken. Gezonde vrouwen gebruiken eveneens dezelfde zones voor fonetische controle. Er was echter een verschil bij het vergelijken van de fonetische taken tussen deze 2 populaties (MTD vs gezond): de groep vrouwen met MTD vertoonde hogere hersenactiviteit in de precentrale gyrus, onderste, middelste en bovenste frontale gyrus, linguale gyrus, insula, cerebellum, middenhersenen en hersenstam en een lagere hersenactiviteit in de cingulate gyrus, bovenste en middelste temporale gyrus en onderste pariëtale hersenkwab. Bij patiënten met MTD, kan deze alternerende (hogere/lagere) hersenactiviteit resulteren in andere laryngale spanning en stemsymptomen.

De studie van CNS stemcontrole bij gezonde zangeressen met supgralottische compressie heeft een significant effect van fonatie controle in zowel de bilaterale pre/postcentrale gyrys, en in de frontale, cingulate, bovenste en middelste temporale gyrus, als in de pariëtale hersenkwab, insula, lingual gyrus, cerebellum, thalamus, en hersenstam aangetoond. Deze geactiveerde gebieden zijn consistent met eerdere onderzoeken die andere fRMI protocollen volgden. Bovendien werd een significant effect van fonatie in de bilaterale bovenste temporale gyrus en de pre/post centrale gyrus gevonden. Bij gezonde zangeressen met supraglottische compressie, kan de hersenactiviteit in de pre/postcentrale gyrus een biomarker zijn van laryngale supraglottische compressie gedurende fonatie.

De grootste uitdaging in het verder onderzoek naar CNS stemcontrole is het aantonen van het verband tussen de routinematige diagnostische gedragsmetingen (i.e. perceptueel, akoestisch en aerodynamisch) en beelden van de hersenen om een beter zicht te krijgen op de relatie tussen de huidige klinische stemmetingen en de onderliggende (pathologische) neurologische aansturing bij de dysfone stem. Daarenboven, zal een studiegroep van neurologen, NKO specialisten en spraakpathologen deze onderzoeken verder moeten ontwikkelen, uitvoeren en analyseren. Een beter begrip van CNS stemcontrole zal helpen om biomarkers te identificeren die kunnen leiden tot betere diagnostiek en uiteindelijk tot het verder aanpassen van de behandeling van de professionele stemgebruikers met dysfonie.

# LIST OF PUBLICATIONS

The dissertation is based on the following manuscripts:

1. Van Lierde K.,* Kryshtopava M.,* Van Maele G., Boehme B., Piens N., Vonck K. Impact of Vagal Nerve Stimulation on Objective Vocal Quality, a Pilot Study. Journal of Voice, 2015, 29(6), 777. e779-777. e715. DOI: 10.1016/j.jvoice.2015.01.010

*equal contribution

2. Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2017,31(1):118. e121-118. e128. DOI: http://dx.doi.org/10.1016/j.jvoice.2016.02.021

3. Kryshtopava M., Van Lierde K., Meerschman I., D'Haeseleer E., Vandemaele P., Vingerhoets G., Claeys S. Brain activity during phonation in women with muscle tension dysphonia: an fMRI study (in press) *Journal of Voice*, DOI: http://dx.doi.org/10.1016/j.jvoice.2017.03.010

4. Kryshtopava M., Van Lierde K., Defrancq C., De Moor M., Thijs Z., D'Haeseleer E., Meerschman I., Vandemaele P., Vingerhoets G., Claeys S. Brain activity during phonation in healthy female singers with supraglottic compression: an fMRI pilot study (submitted in 2017) *Logopedics, phoniatrics, vocology*

# ABBREVIATIONS

3D MPRAGE: three-dimensional magnetization-prepared rapid acquisition with gradient echo

ACC: anterior cingulate cortex

BA: Brodmann's area

BOLD: blood oxygenation level dependent

CMT: circumlaryngeal manual therapy

CNS: central neural system

CPG: central pattern generator

CT: cricothyroid

DSI: dysphonia severity index

EMG: electromyography

ENT: ear, nose and throat

F0: fundamental frequency

FDR: the false discovery rate

fMRI: functional magnetic resonance imaging

FWHM: full width at the half maximum

GLM: general linear model

HNR: harmonics-to-noise ratio

HRF: hemodynamic responses function

ICA: Independent Component Analysis

IFG: inferior frontal gyrus

LEMG: laryngeal electromyography

LMC: laryngeal motor cortex

LPR: laryngopharyngeal reflux

LV: laryngeal videostroboscopy

M1: primary motor cortex

MDVP: Multi Dimensional Voice Program

MFG: middle frontal gyrus

MH: mylohyoid

MPT: maximum phonation time

MTD: muscle tension dysphonia

MTG: middle temporal gyrus

NTS: nucleus tractus solitarius

PAG: periaqueductal gray

PET: positron emission tomography

PRT: Brain Voyager protocol file

QOL: quality of life

R1: first acoustic resonance of the vocal tract

RF: reticular formation

RFX-ANCOVA: ANCOVA Random Effect Analysis

RLN: recurrent laryngeal nerve

S1: primary sensory cortex

SD: spasmodic dysphonia

SFG: superior frontal gyrus

SH: sternohyoid

SLN: superior laryngeal nerve

SMA: supplementary motor area

Spt: planum temporale

STG: superior temporal gyrus

TH: thyrohyoid

VC: vital capacity

VF: vocal fold

VHI: voice handicap index

VN: vagal nerve

VNS: vagal nerve stimulation

VTC: volume time course

# CHAPTER 1

# INTRODUCTION

The prevalence of functional dysphonia in the working-age population (25 – 64 years) seeking consultation in an ear, nose and throat (ENT) department is 41%, and female professional voice users are predominantly affected (43% women vs. 36% men)[1]. The term muscle tension dysphonia (MTD) is often used to describe functional voice disorders with vocal hyperfunction. It is caused by dysregulated laryngeal muscle activity, or excessive muscle usage in phonation[2,3]. Causes of MTD include environmental (external) or systemic (internal) factors or stimuli, such as upper respiratory infection, second-hand smoke, laryngopharyngeal reflux (LPR), significant vocal demands, or stressful life events[4].

Recent studies, however, have demonstrated that laryngeal compression may be a regular laryngeal behavior during normal speaking and singing [5-13]. Thus far, no studies have been able to identify neural biomarkers that indicate supraglottic compression and may help to determine whether laryngeal compression is either a dysfunctional or a normal laryngeal behavior.

Additionally, a voice disorder that is caused by vagal nerve stimulation (VNS) can be distinguished from the category of functional voice disorders. VNS is most often used to treat epilepsy when other treatments are not effective. It involves delivering electrical impulses to the vagus nerve (VN). However, VNS is often associated with alteration of voice (66%) [14]. This voice disorder may be caused by the impulses from the electrode that not only go to the brain, but also travel to the superior laryngeal nerve (SLN) and recurrent laryngeal nerve (RLN), and then to the larynx[15]. Study of the impact of VNS on the vocal characteristics presents a unique possibility to assess alteration of voice by mimicking functional voice disorders developed as a result of stimulating the SLN and/or the RLN and thereby exciting either the afferent and/or efferent nerve fibers of the laryngeal system.

The neurophysiological background of functional voice disorder is not fully understood [16-20]. More specifically, the role of central neural system (CNS) control of voice in patients with functional voice disorder is currently unknown. In fact, even in healthy people, the neurophysiological mechanisms of how brain controls phonation are practically unknown.

# 1. Voice and voice disorders

## Voice, vocalization, phonation, speech, singing... What's the difference?

Definition of voice, vocalization, speech and phonation are often used interchangeably, but there are differences between them. Voice is defined as a production of sounds during phonation for crying, humming, speech, and singing[21]. Voice is present at birth and becomes further differentiated as the infant develops and begins to speak. Speech is a learned vocal behavior that conveys meaning and involves the formulation of meaningful phrases through lexical selection and grammatical relationships requiring language processing. Human singing is also a learned vocal behavior that can be produced both with and without semantic content. The basic activity of all these processes (i.e., voice, speech, singing, and vocalization) is phonation. Human phonation is a laryngeal motor behavior that extends from reflexive and unlearned limbic laryngeal actions,[22-24] to controlled and coordinated, highly skilled vocal movements that support speech and/or singing [25, 26]. Phonation is developed through a gradual process of increased adaptation resulting in a more complex behavior[25].

## Voice disorders

A voice disorder is characterized by the abnormal production and/or absence of vocal quality, pitch, loudness, resonance, and/or duration, which is inappropriate for an individual's age and/or sex[27]. The underlying cause of a voice disorder can be organic or functional. Organic voice disorders result from acquired morphological changes of the vocal cords (e.g. cysts, nodules, papilloma, polyps) of the larynx. However, a functional voice disorder occurs when vocal quality deteriorates in the absence of anatomic and neurological factors. Most of functional disorders fall under the category of MTD. In addition, the functional group includes a voice disorder caused by VNS[14, 28, 29].

### *Muscle tension dysphonia*

MTD is a common functional dysphonia that is clinically characterized by vocal hyperfunction [16, 17, 30-32]. Vocal hyperfunction can be defined as the involvement of too much muscle force and physical effort during phonation[2,3].

MTD is often observed in vocally untrained people who have used their voices extensively[30]. The main symptoms of MTD include dysphonia, often with limited vocal pitch ranges (such as a high and narrow vocal pitch interval) [33-37], neck or shoulder stiffness, excessive vocal effort, vocal fatigue, and unpleasant sensation in the larynx (discomfort, laryngeal pain and/or tiredness) [16, 17]. All of these symptoms intensify with extended vocal use. While it is often associated with the absence of *primary* organic (primary MTD) or neurologic laryngeal disorders, MTD may lead to organic pathologies (secondary MTD) such as secondary vocal nodules that require complicated and costly surgeries[20, 38].

The main features of MTD include laryngeal elevation during phonation (the hyoid and larynx are in a higher position in MTD patients compared to controls) [35], abnormal glottal closure, excessive supraglottic activities (anteroposterior contraction and ventricular fold adduction) (Figure 1.1), and increased external laryngeal muscle activity [30, 39].

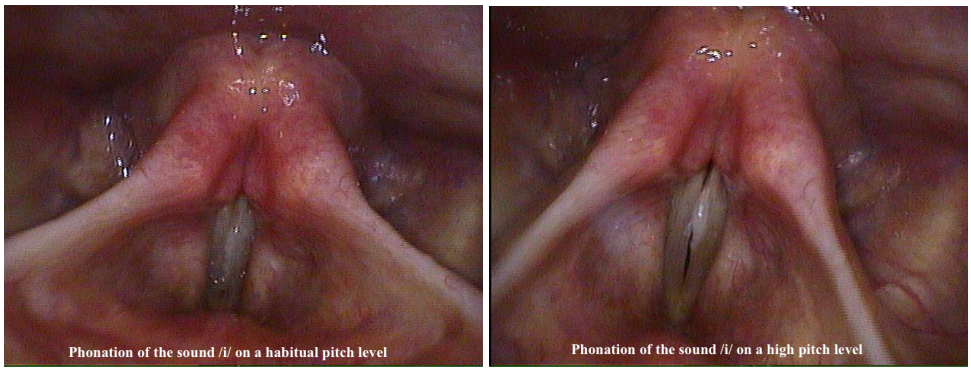


**Figure 1.1: Larynx in MTD.** Complete glottal closure was defined during phonation of the sound /i/ on a habitual pitch level. Incomplete glottal closure was defined during phonation of the sound /i/ at a high pitch level.

Increased posterior cricoarythenoid muscle activity and lateral supraglottic contraction during phonation of the sound /i/ at a high pitch level. Causes of MTD include environmental (external) or systemic (internal) factors or stimuli. The most common factors or stimuli are upper respiratory infection, second-hand smoke, LPR, significant vocal demands, or stressful life events[4]. Most often, more than one factor is likely to be involved. In MTD, hyperfunctional vocal behavior is often a result of inappropriate compensatory strategies for muscle activities that are adopted in response to these sensory perturbations (i.e., environmental or systemic stimuli)[20].

Patients with functional dysphonia have also been characterized as introverted, stress reactive, alienated, and unhappy[40, 41]. The reaction of the individual to stress may vary according to their personality characteristics[40, 41]. It may be only hypothesized that the reaction of the brain to external or internal stimuli may vary according to personality characteristics and/or behavior.

The pathophysiology of MTD is not fully understood [16-20]. The major pathophysiological finding in patients with functional voice disorders has been that the hyoid and larynx positions are higher in such patients than in healthy controls [35]. The only muscles which may be affected in this context is the thyrohyoid (TH) muscle which raises the larynx to the hyoid, the anterior belly of the digastric muscle and the mylohyoid (MH) [42] muscle in the submental region which pull the hyoid upwards. Van Houtte et al [18] have found TH muscle overactivity during phonation in patients with MTD compared to a healthy group. However, no studies have verified that the anterior belly of the digastric muscle and the MH muscle are consistently activated in MTD. The MH muscle is deep under the platysma, sternohyoid (SH), and the omohyoid and cannot be accurately recorded from the neck surface by using surface electromyography (EMG). To our knowledge, there have been no studies using intrinsic laryngeal electromyography to study the activation levels of any of the intrinsic muscles such as the anterior belly of the digastric, MH, thyroarythenoid, lateral cricoarytenoid, cricothyroid or posterior cricoarytenoid muscles in patients with MTD. Most of the recent studies aimed at determining biomarkers of functional dysphonia have used paralaryngeal surface electromyography, mechanomyography, high-resolution manometry, and 24-h Dual-probe pH-metry [18-20, 43-48]. These studies, however, do not provide any evidence for a primary biomechanical disturbance in patients with voice disorders; no significant differences in phonation-induced

upper esophageal sphincter pressure and in electromyographic activity of submental, infrahyoidal, and sternocleidomastoid muscles between MTD patients and normal speakers have been detected [18, 19, 49]. The surface electromyographic measures that have been taken from the anterior neck region and were able to measure the platysma and SH muscle, neither of which is consistently active for phonation. The SH muscle pulls the hyoid downwards towards the sternum and has not been postulated to be overly active in MTD.

Current treatment of MTD is based on alleviating the local causes by using indirect voice therapy (i.e. patient education and vocal hygiene), direct voice therapy, medical treatment, and surgery (e.g. secondary vocal nodules). Direct voice therapy includes working on posture, breathing, phonation, articulation, working on muscle tension using progressive relaxation [50], and vocal facilitation techniques (chewing exercise[51], yawn-sigh approach[52], biofeedback training [53] and circumlaryngeal manual therapy [CMT][54]). Unfortunately, this approach is not effective for a significant proportion of MTD patients[55]. The reported success of voice therapy varies between 64% [54] and 90% [56]. According to Speyer [57],the literature on the effects of voice therapy in dysphonic patients is limited and many studies have methodological problems. For example, in case of perceptual evaluation, it is very often unclear whether the data have been offered to the listeners in randomized order and the reports often lack information on pre- or posttherapy status of the voice samples. Some studies use very subjective instruments to evaluate therapy effects without any statistical foundation. Furthermore, the lack of a good group of healthy controls receiving no therapy weakens many study designs. Usually, the results of the effect studies are based on small or restricted groups of patients and a small number of speech therapists. Often only restricted sets of assessment instruments have been used in the experiments. As a consequence, many aspects of voicing may be overlooked.

### *Voice disorder induced by vagal nerve stimulation*

VNS is a medical treatment of patients with epilepsy that is sometimes employed when other treatments have failed. It involves delivering electrical impulses to the VN which results in further dissemination of a low-frequency electric pulse from the VN toward the CNS. In this procedure, an electrode is implanted in the neck around the left VN and activated by a pulse generator implanted

subcutaneously in the left infraclavicular region (Figure 1.2). Both the frequency and the amplitude of the stimulus can be programmed individually in order determine a level of treatment that may lead to a decrease in the frequency and severity of epileptic seizures [58]. It is also sometimes used as an adjunctive treatment for certain types of intractable epilepsy and treatment-resistant depression. However, VNS causes simultaneous stimulation of the SLN and RLN and is associated with problems ranging from alteration of voice (66%), coughing (45%), pharyngitis (35%),throat pain (28%), [14] and hoarseness (very common) to frank laryngeal muscle spasm and upper airway obstruction (rare) [59]. "Increased muscle tension," presumably in the upper body, may be experienced during the stimulation period [60]. Voice disorder induced by VNS is considered secondary to peripheral sensory perturbations as a result of prolonged VNS[61]. However, the underlying pathophysiological mechanism is not yet well-understood.

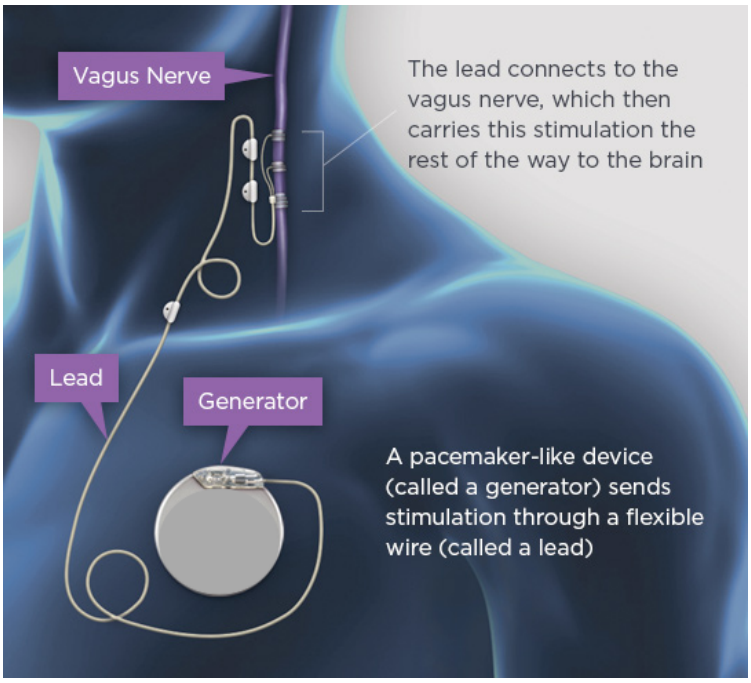


**Figure 1.2: VNS therapy (modified from a model proposed http://us.livanova.cyberonics.com/vns-therapy/how-vns-therapy-works).**

## 2. Central nervous system control of voice and voice disorder

### Central nervous system control of voice

Voice control by the human CNS is unique as this innate system is intact at birth and followed by adaptation of voluntary control of voice. In order to identify regions of the brain specifically involved in vocalization, Brown et al[62] conducted a meta-analysis of phonation (syllable singing), which was then compared with Turkeltaub et al[63] meta-analysis of word production. Results of this meta-analysis showed significant areas of overlap in the larynx motor cortex, supplementary motor area (SMA), the rolandic operculum, superior temporal gyrus (STG) and cerebellum. However, there were also areas where activation was specific to syllable singing compared to overt reading in the frontal operculum, STG, putamen and thalamus. These results help to identify the specific brain regions associated with models of voice control. The authors suggest a neural model of vocalization in which the principal regions for the control of phonation in speaking and singing are the STG, the larynx motor cortex (LMC) and associated premotor areas, the cerebellum and the SMA. These data show similarities with the "basic speech production network" proposed by Bohland and Guenther[64] where it is suggested that additional sequence and syllable complexity leads to increased engagement of this speech network and recruitment of additional brain areas. It may be expected that vocalization will involve a reduced engagement of this network when compared to more complex speech. During vocalization the vocal-sensorimotor system provides both somatosensory and auditory feedback, which is used to compare actual and intended vocal output to regulate voice fundamental frequency (F0) through error-induced corrective commands. The model specifies that auditory error cells locate in the STG and respond when a mismatch between the auditory feedback signal and the auditory target is detected. The projections from the auditory error cells transform the auditory error into a motor command to correct voice F0 to match actual vocal output with planned vocal output. Results from this study also showed increased BOLD in the STG regions when comparing the fixed effects group results of shifted vocalization versus non-shifted vocalization. Further evidence of the involvement of the STG in a feedforward system to control vocal output is provided by Tourville et al[65], who used fMRI to examine formant-shifted speech during production of monosyllabic words to test the

DIVA model predictions of brain areas involved in articulatory control. The DIVA model of speech production[66-68] is another model that incorporates auditory feedback and feedforward commands for voice and speech control[69].

In this study, we use a neural model of human phonation that consists of (1) the vocal motor control system [70, 71], (2) the reflexogenic control system [22], and (3) the feedback control system[69, 72-78] (Figure 1.3). We use this model of human phonation to explain in a simplified way the complex network of human vocal behavior presented by Ackerman et al [79, 80]. The vocal motor control system is responsible for laryngeal motor control while the other two systems maintain laryngeal sensory control during phonation. Interaction and seamless cooperation of these basic neurological control systems is indispensable for normal phonation.

### *Vocal motor control*

The vocal motor control system is responsible for laryngeal motor control. It has a three-level organization from the brainstem to LMC[81] and consists of two parallel pathways[70, 82] (Figure 1.3). The first pathway is derived from the anterior cingulate cortex (ACC) and controls innate and emotional vocalization (i.e. unlearned limbic vocalization), such as crying, groaning or laughing, whereas the second pathway is originated from the LMC and controls learned vocal behaviors, such as speech and singing [83, 84].

The highest level of the human vocal motor control neural network is the LMC (located in the primary motor cortex) [62, 85-88] and its modulating systems (such as the inferior frontal gyrus [IFG] (i.e., Broca's area), cerebellum, SMA, STG, and supramarginal gyrus) (Figure 1.3) [70, 87-92]. In humans, the LMC establishes both the direct [89, 93] and indirect (via the reticular formation (RF)) [82] connections with laryngeal motoneurons which are responsible for control of laryngeal movements for learned but not innate vocalizations (Figure 1.3)[82]. The LMC is also responsible for the integrative control of breathing during voice production for speech and singing[88, 92, 94].

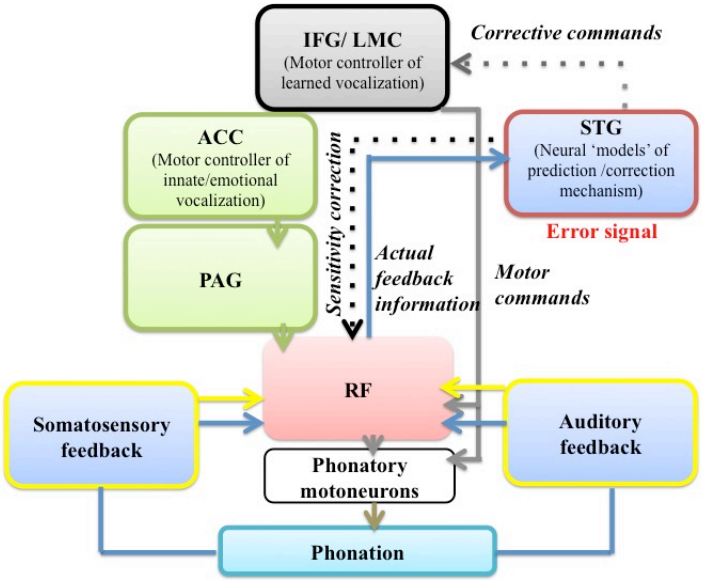


**Figure 1.3: Schematic diagram of laryngeal neural control of normal phonation (modified from a neural model of vocalization proposed by Zarate[61]).** The vocal motor control system (central columns), reflexogenic system (yellow-outlined boxes and yellow arrows), and feedback system (blue boxes and arrows). The lower level of the vocal motor control system, the reticular formation (RF) (red box), generates complete vocal patterns to phonatory motoneurons (white box). The middle level of the motor control system, the anterior cingulate cortex (ACC) and periaqueductal gray (PAG) (green boxes), guides emotional vocalization. The upper level of the laryngeal motor control system, the laryngeal motor cortex (LMC), is responsible for producing learned/skilled vocalizations (i.e., speech and song) and requires inputs from the inferior frontal gyrus (IFG) for motor planning of voice (other modulatory brain regions of the LMC are not depicted) (gray box). Feedback from phonation is processed by the ascending somatosensory (left) and auditory (right) pathways and transmitted to the superior temporal gyrus (STG) (blue boxes and arrows; only selected regions of these pathways are shown) via the RF (red box). Sensory feedback from phonation provides actual information (how it feels), while the STG (red-outlined box; other possible brain regions involved in the prediction/correction mechanism are not depicted), provides information on the expected state (how should it feel) relying on a neural 'model'. The mismatch between actual sensory feedback and sensory predictions of motor commands indicates an error signal that, if large enough, would trigger changes in the neural 'model' generating alterations in motor control (sending corrective commands (gray dotted arrow)) and sensory perception (changing sensitivity (black dotted arrow)).

The middle level of the vocal motor control system is located in the ACC and the periaqueductal gray (PAG) (Figure 1.3). The ACC and PAG guide the phonation for innate and emotional vocalization[70, 83, 84, 95, 96]

The lower level of the vocal motor control neural network is the RF of the brainstem (Figure 1.3). The RF contains a central pattern generator (CPG) for vocalization which generates motor patterns of laryngeal muscle co-activation [97-99]. However, most of the research on the brainstem network was conducted in animal studies [83, 96, 100, 101] rather than humans [102, 103].

Recent studies have proposed potential integrative neural substrates linking innate and learned vocal neural pathways: the ACC [95, 104, 105], basal ganglia [80, 95, 106], cerebellum [79, 95, 105, 106], and auditory cortex [77, 104, 106]. However, most of these studies have focused on the affective vocalization control [95, 104-106] and articulatory control [79, 80] rather than on laryngeal neural control[77] of phonation.

### *Vocal sensory control*

Phonation control depends on sensory input that provides the target for laryngeal motor control of phonation. Sensory control includes the reflexogenic and feedback systems.

The reflexogenic system fine-tunes vocal fold movements and relies on three components of a reflex arc: the sensory afferents (laryngeal, pulmonary, esophageal, and cochlear), brainstem function, and laryngeal efferents (Figure 1.3). Wyke [22] has divided the reflexogenic system into two categories: intrinsic and extrinsic.

The intrinsic reflexogenic system is elicited by stimulation of the laryngeal afferents contained in the SLN [107-110]. Subglottic air pressures, stretching forces, and tension in the laryngeal joints stimulate these afferents. The laryngeal afferent information is projected to the nucleus tractus solitarius (NTS) and the RF in the brainstem [108]. These structures process incoming information and exert coordinated excitatory and inhibitory influences on the laryngeal efferents in the nucleus ambiguous [22, 111-115]. Coordinated excitatory

and inhibitory influences on the laryngeal efferents adjust reflexive vocal fold movements thus maintaining normal phonation [22].

The extrinsic reflexogenic system is related to the pulmonary, esophageal, and auditory reflexes. The auditory reflexes (i.e., "Lombard" reflex/effect, middle-ear, and olivocochlear acoustic reflexes) make continuous corrections for sound perception and vocal output [116-121]. In 2006, Hage et al [121] discovered audio-vocal neurons in the RF that play a crucial role in the gating of incoming sounds for the auditory reflexes. The pulmonary [22] and esophageal [122] reflexes have no influence on the phonatory activity of the laryngeal muscles in healthy people.

The second sensory system, feedback, plays a key role in development of phonation[123, 124] for *learned* vocalization. The feedback system includes two forms: auditory and somatosensory feedback [69] (Figure 1.3). During human development, feedback from motor and sensory systems [77] provides information necessary for adaptation of phonation and particularly of vocal quality to the continuously changing environment. Without sensory information phonation does not develop normally [25]. Specific brain regions are involved in the control of the sensory feedback of vocalization: the STG[77,125], planum temporale (Spt), primary and secondary somatosensory cortex, as well as the insula[71]. Moreover, studies by Parkinson et al[77] have shown STG activity as a key region involved in processing auditory and somatosensory feedback of vocalization.

From more fundamental neurobiological point of view, the modulation to sensory feedback brings about significant central neuroplastic changes [126, 127]. Neural plasticity or brain plasticity is the ability of the CNS to change and adapt in response to sensory stimuli, environmental cues, experience, behavior, injury or disease by reorganizing its structure, function and connections [126, 128, 129]. Neural plasticity can result from a change in function within a particular neural substrate in the CNS through alterations in neuronal excitability[130]. Changes in the function of a neural substrate can then alter behavior secondary to environmental influences such as experience, learning (e.g. vocal training may result in changes in laryngeal activity)[131], development, aging, change in use, injury or response to injury such as unmasking due to the loss of surround inhibition with reduced afferent input[132-134]. Neural plasticity may alter the

function of the original neural substrate used to produce a regular behavior[135]. Understanding how the brain adapts to a changing environment will provide insight into how this adaptation influences the development of phonation and its disorders.

### *Vocal learning and adaptation*

During development of phonation and particularly of vocal quality, laryngeal control becomes increasingly skilled and rapid. Moreover, the balance of aerodynamic and muscle forces adapts to rapidly changing vocal requirements, including modulations of pitch, loudness, and rate. Integration of the sensory input and laryngeal motor output is required for this adaptation [75, 136, 137]. However, sensory feedback control is too slow to support the required rapid and skilled vocal movements. Most of these movements are pre-programmed. These programs require the generation of internal representations or a neural 'model' of the sensorimotor transformations required to generate the set of motor commands that will execute a desired movement. This neural 'model' reinforces or corrects the motor activation in the brain [74] and adjusts brain processing to the current sensory information [138] (Figure 1.3). Once this neural 'model' is learned, the internal system can then predict likely sensory consequences of a motor command prior to the arrival of actual sensory feedback. Thus, online feedback control of phonation is achieved primarily via the neural 'model' whereas actual feedback information is used to train and update this neural model. Actual feedback provides necessary information and plays a key role in learning, maintaining, and updating the neural 'model' and can also be used to correct overt prediction/feedback mismatch errors[75]. Any changes in the larynx require adaptation and updating of this neural model[74]. There have been many studies that attempt to establish the neural correlates of the neural 'model'[139, 140]. The premotor cortex [141, 142] and cerebellum [65, 143] are involved in the control of the neural 'model'. The STG has been identified as an integration area of sensory input and motor output during phonation[75, 77], specifically during error detection and correction involved in pitch processing[65, 66, 77, 144, 145]. Furthermore, the STG is involved in auditory-vocal integration and processing of predicted and actual vocal output[146]. Brain imaging studies have shown that some areas of the brain are more active when unexpected sensory perturbation is present. Studies by Parkinson et al[147] have shown that STG is a potential key brain area activated during vocalization with changed feedback. A functional magnetic resonance imaging (fMRI) experiment by Parkinson et al[147]

identified STG activation during pitch-shifted (compared to non-shifted) vocalization and did not reveal activation during non-shifted vocalization compared to pitch-shifted vocalization. It has been suggested that a match between expected and actual output results in suppression in the auditory cortex and leads to an overlapping pattern of activations. On the other hand, a mismatch between expected and actual output results in an increase of sensitivity in the auditory cortex [148, 149].

## Central neural system control of functional voice disorders

The neurophysiological background of MTD is not fully understood [16-20]. The absence of an appropriate model that is truly representative of human vocal learning and adaptation is largely responsible for the latency in research of CNS control of voice.

### *Vocal motor control of functional voice disorder*

The vocal motor control system transmits impulses to the motoneurons that control movements of the [150]laryngeal muscles, thus enabling maintenance of balance in the larynx during phonation. Recent experimental studies have shown that complex vocal fold alterations (inflammation and trauma) and sensory stimulations cause tic changes with prolonged excitability in the divisions of the vocal motor control system: the PAG and RF [107,151,152]. These regions are responsible for coordination of phonatory motoneurons via CPG for vocalization [83, 97, 153]. However, most research on the brainstem network has been conducted in animal studies[83, 84, 96, 100, 101] rather than in humans[102, 103]. Morrison et al[154,61] have hypothesized that repeated stimulation of the laryngeal sensory afferents by noxious stimuli may result in a hyperexcitable state of the laryngeal muscle. Morrison et al[154,61] have also hypothesized that emotional or sensory triggers cause neuroplasticity in the PAG that results in laryngeal motor control alteration via projections to the RF which contains the CPG for vocalization. However, currently no known neurophysiological mechanisms are available to support this hypothesis.

Evaluation of VNS presented a unique possibility to assess the neurobiological basis of CNS modulation during phonation. Major challenges in this field include the absence of animal models of real-life speaking/voicing and a limited range of noninvasive studies that can be performed in humans to assess

the neural bases of this complex behavior. Humans remain the only species that can be studied in methodologically demanding experiments to assess sensorimotor cortical control of phonation for voluntary learned vocalization. VNS has recently become an object of study in regulating cortical plasticity[150, 155]. Moreover, the larynx is mainly innervated by the SLN and RLN from the VN. In the brainstem, the sensory afferent fibers terminate in the NTS, which then send fibers that connect directly or indirectly to different brain regions. These regions include the dorsal raphe nuclei, locus coeruleus, amygdala, hypothalamus, thalamus, periaqueductal gray, the anterior cingulate cortex, and orbitofrontal cortex. Functional MRI studies have reported activation of these areas during VNS [156-173]. Many of these regions respond to and modulate phonation, including the insula, PAG, ACC, somatosensory cortices, thalamus, and prefrontal cortex.

Voice disorder induced by VNS is considered secondary to peripheral sensory perturbations as a result of prolonged VNS[61]. The VN projects toward central brainstem structures, such as the NTS, locus coeruleus, and RF to other limbic, sensory, cortical, and subcortical areas. The transmission of electric stimuli through these neuronal projections provides direct modulatory effects in subcortical sites and changes in cortical excitability. This voice disorder may develop as a result of stimulating the SLN and/or the RLN and thereby exciting either the efferent and/or nerve afferent fibers of the laryngeal system. The RLN innervates all laryngeal muscles except the cricothyroid (CT) muscle, which receives its motor impulses from the external division of the SLN. Stimulation of the RLN may induce hypertension of the laryngeal muscles during VNS. Laryngoscopy and videostroboscopy have shown left vocal cord adduction during stimulation at 30 Hz and higher [174], vocal fold tension[15, 29], loss of mucosal wave[15], supraglottic muscular hyperfunction, and reduced vocal fold mobility[29] as the most common findings during VNS activation. However, the precise mechanisms by which the CNS exerts its effects remain unknown. A study focused on the VN as a possible conduit for sensory stimuli pertinent to phonation control may present a unique possibility to assess the influence of vocal adaptation on structure and function of the human brain. Using VNS as a human model of real-life vocalization may illustrate a correlation between sensory stimuli, vocal changes, and vocal control.

### *Vocal sensory control in voice disorders*

Numerous reflexes affect laryngeal muscle control [23, 175]. Laryngeal hyperfunction may represent an abnormal excitation and/or reduced inhibition of the laryngeal adductor response [176], the esophageal [122] and the pulmonary reflexes [22]. However, as there is no evidence of increased tension in the intrinsic laryngeal adductor muscles in patients with MTD. The only muscles which could likely be stimulated in MTD are the anterior belly of the digastric and MH muscles[35] as well as the TH muscle. Laryngeal reflexes do not activate these muscles; there is no basis to suggest that disturbed reflexogenic control may contribute to hyperfunctional vocal behavior in functional voice disorder.

Feedback plays an important role in development of phonation. Any change in the larynx results in changes of voice. Moreover, healthy women have greater sensitivity of the larynx than men [177] and may rely more heavily on laryngeal sensory inputs for voice control [178]. Therefore, even small changes in afferent innervation may result in voice disorders. However, no neurophysiological mechanisms are currently available to support these suggestions. In MTD, the sensory stimuli associated with phonation are altered, such as poor vocal quality, upper respiratory infection, LPR, vocal demands, and life stress, and may trigger changes in laryngeal neural control of phonation and alter muscle activation patterns. However, the precise neurophysiological mechanisms by which the CNS exerts its effects on laryngeal neural control of phonation remain unknown.

Stimulation of the SLN during VNS may alter the brain activities of phonation via stimulation of vagal *afferents* and their connections in the brainstem and forebrain. Internal division of the SLN is a branch of the VN that provides laryngeal *sensory* innervation. The afferent fibers of the SLN travel along the VN in the carotid sheath. Voice disorder induced by VNS is considered secondary to peripheral sensory perturbations as a result of prolonged VNS[61]. VNS may intervene in the sensory feedback system and modulate laryngeal sensorimotor responses resulting in altered autonomic balance and laryngeal hyperresponsiveness. Evidence has been reported suggesting that VNS causes long-term neuroplasticity in the brain[179]. Neuroimaging studies corroborate these effects, showing neuronal activity changes in certain sites within the brain includingthe amygdala, insula, precentral gyrus, hippocampus, and thalamus [168, 180-

[182]. These neuroanatomical connections have been linked to the "bottom-up" mechanism of modulation by CNS [183]: the transmission of electric stimuli follows an inverse path from peripheral nerves toward the brainstem and central structures.

### *Vocal learning and adaptation in voice disorders*

Recently an association between the mechanism of vocal learning and adaptation and symptoms of MTD has been described[184]. However, there are no studies that have evaluated neural correlates of phonation in MTD and its association with vocal learning and adaptation. The hypothesis that MTD is caused by disruptions to the internal model resulting from altered auditory and/or proprioceptive feedback was suggested by Urberg-Carlson[184, 185]. This hypothesis suggested that in MTD the sensory stimulation associated with phonation that are altered, such as poor vocal quality, upper respiratory infection, LPR, vocal demands, and life stress, may trigger changes in the neural 'model' of the sensorimotor transformations that are required to generate the set of motor commands. This altered sensory stimulation induces an error signal between the actual sensory information and its prediction. The error signal, if large enough, would trigger changes or updates in the neural model, and would in turn generate corrective commands to the motor controller as well as alter sensory perception. The updated or new neural 'model' may support the symptoms of MTD by altering motor cortical commands in the areas responsible for motor control and by changing sensory perception in the areas responsible for sensory control of phonation. In this case, altered descending motor cortical signals stimulate laryngeal motoneurons in the brainstem that might result in excessive tension of [150]laryngeal muscles or recruit muscles that are not ordinarily active. Simultaneously, altered sensory perception makes the brain insensitive to normal feedback even when irritants are no longer present. Callan, et al[186] showed that a DIVA model[69] that incorporates adaptive vocal learning is able to adapt when changes are made to its "vocal tract". Schiller et al[187] and Nasir and Ostry[188] have shown that motor learning produces changes in the boundaries of perceptual targets. Furthermore, Schiller et al[187] showed that motor learning enables acceptance of the altered feedback as if it were an accurate production. This suggests that feedback may plastically reduce the impact of the error signal, which may lead to a situation in which it is no longer recognized as an error signal. In this situation, the updated or changed neural 'model' could begin to predict dysphonia as a result of the motor commands.

The error signal is no longer produced because the resulting productions match the expected signal. The motor command that results in dysphonia is therefore maintained. However, currently known neurophysiological mechanisms supporting this hypothesis remain unavailable.

## 3. Clinical Assessment of Voice

### Voice assessment protocol

Clinical examination of voice requires a voice assessment protocol (VAP). The current VAP includes five domains: (1) auditory-perceptual measures, (2) voice range, acoustic and aerodynamic analysis of the voice, (3) dysphonia severity index (DSI) measurements, (4) visualization of the vocal folds and (5) patient self-assessment. A patient with MTD typically undergoes assessment in each of these five areas. Voice range, aerodynamics, acoustic, and DSI measurements are objective assessment techniques. Generally, MTD leads to deviations from normative measures of the VAP due to increased laryngeal tension. The altered data within the VAP include reduced vocal range, reduced aerodynamics (maximum phonation time [MPT] and and vital capacity [VC]), increased jitter and shimmer, and reduced DSI[18, 19]. The assessment methods for laryngeal muscle activity include palpation, musculoskeletal assessments [189], fiberoptic nasolaryngoscopy [190, 191], and surface EMG [18]. Currently, the diagnosis of MTD is made based upon multidimensional voice testing, and is made based on agreement between the voice therapist and otorhinolaryngologist. However, there remain no specific biomarkers of MTD that determine supraglottic compression and help to understand whether laryngeal compression is either a dysfunctional or a normal laryngeal behavior. A better understanding of CNS control of voice may help to establish biomarkers of MTD.

## 4. Neuroimaging evaluation of voice

The CNS control of voice and speech is best determined using human brain imaging technology to identify the neural substrates involved[192]. Methods such as fMRI and positron emission tomography (PET) can be used to determine the neuronal substrates involved in phonation and how these neural substrates can

be modified through learning, development, aging, and following disease. fMRI is a non-invasive objective tool which reflects changes in neuronal firing within neural substrates by quantifying blood oxygenation level dependent (BOLD) changes. fMRI has become an important tool to describe neural networks associated with laryngeal control of phonation for voice, speech, and/or singing [88, 193-196]. Previous studies have identified the sensorimotor cortex region (corresponding to Brodmann's area (BA) 1, 2, 3, or 4), premotor cortex region (BA 6, 8), STG (BA 22,41, 42), insula (BA 13), cingulate gyrus/cortex, supramarginal gyrus (BA 40), lingual gyrus (BA 18, 19), thalamus, cerebellum, midbrain, and basal ganglia as key regions in the functional network of non-disordered phonation [62, 88, 193-195, 197](Figure 1.4). More specifically, as defined by functional brain imaging, the sensorimotor cortex region functionally includes the primary motor cortex (or M1) (BA 4) and the primary somatosensory cortex (or S1) (BA 1, 2, and 3), and is anatomically located on/in the pre/postcentral gyrus and central sulcus [198]. In addition, the premotor cortex region functionally includes the premotor cortex as well as the SMA and is anatomically located on/in the precentral gyrus, superior/middle frontal gyrus (SFG, MFG), and IFG [198]. More specifically, the MFG and IFG are responsible for vocal motor planning [199-201]. The IFG is a key region involved in laryngeal *motor* control of vocal pitch modulation[202]. The MTG and STG are responsible for vocal self-monitoring [90] and voice processing[203], respectively. The sensorimotor and premotor cortex regions, STG and insula have been identified as key areas involved in integration of sensory input and laryngeal motor output during vocalization [65, 73, 145, 204]. In addition, the insula is implicated in vocal monitoring as well as detection[205] and integration of sounds with a speaker's emotions and attitudes[206]. Cingulate cortex activity is associated with motor control [174] necessary for phonation, especially during pitch modulation. The cerebellum is involved in motor planning and coordination[207]. Bilateral activations in the precentral gyrus are related to the larynx/phonation motor control area, as described by Brown et al[62].

Lateralization was first discovered in the 1800's by physicians Broca and Wernicke[208]. They identified particular areas of the left hemisphere that play a primary role in speech production. Since that time, bilateral hemispheric involvement has been consistently reported for less complex laryngeal behaviors, e.g., production of voice, coughing, sniffing, voluntary breathing[88, 94, 209, 210]. In 2009, Simonyan et al[92] identified structural and functional brain networks originating from the activation peaks in the primary motor cortex during production of voluntary voice and controlled breathing in healthy humans.

Their major finding was the left-hemispheric lateralization of functional networks during voice production but not breathing despite the presence of largely symmetrical bilateral hemispheric activation during both behaviors and similarly distributed motor cortical structural networks associated with these behaviors.

Neuroimaging studies have shown that singers and non-singers recruited similar brain areas in simple singing, i.e. bilateral auditory cortices, cingulate cortex, supplementary motor area, M1, S1, premotor cortex, insula, thalamus, and cerebellum [145]. The primary motor cortex, supplementary motor area, cingulate cortex, and inferior parietal lobe are key regions in the functional network required to sing single notes [211], melodies [212], or an Italian aria [213].

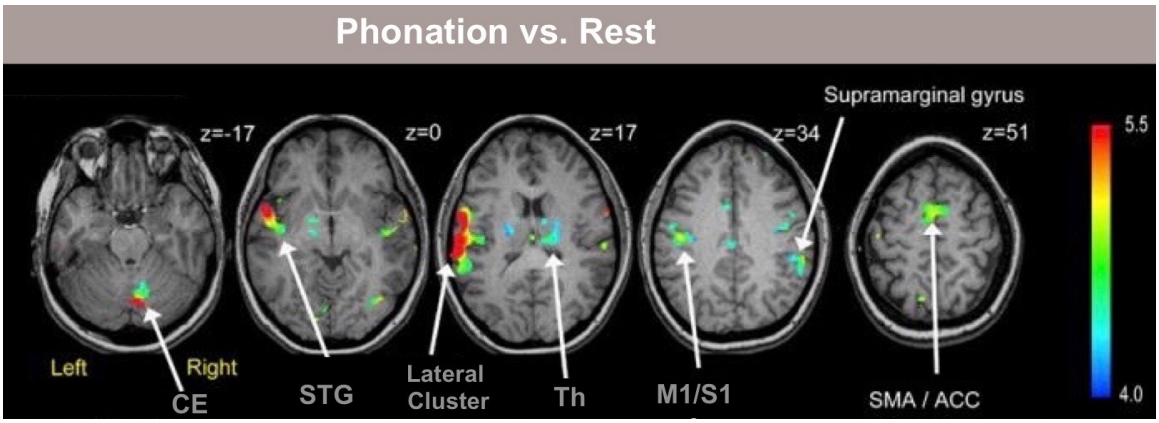


**Figure 1.4: FMRI activations for phonation (modified from Loucks et al, 2007 [79]).** Talairach template brain, z coordinates are given below each slice ($p<0.01$). Prominent activation is found in the left lateral cortex extending from the IFG, through the postcentral gyrus to the STG (BA 1–4, 6, 22, 44 ($z = 17$)), the right cerebellum ($z = -17$), the right supramarginal gyrus (BA 40; $z = 34$), the bilateral pre/postcentral gyrus (BA 3, 4, 6) in a region superior to the left ventrolateral cluster ($z = 34$), the SMA (BA 6, $z = 51$) and extended into the ACC. Prominent subcortical activation is found in ventral and medial nuclei of the right thalamus ($z=18$). (Abbreviations: BA: Brodmann area; IFG: inferior frontal gyrus, SMA: supplementary motor area; M1: primary motor cortex; S1: primary sensory cortex; ACC: anterior cingulate cortex).

An fMRI study by Loucks et al [88] demonstrated that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation. Only a difference in STG activation was seen due to the auditory feedback.

The use of imaging to study voice disorders has mostly been limited to a few specific voice pathologies such as spasmodic dysphonia (SD) [193, 214-217], Parkinson's disease[218-220], and idiopathic unilateral vocal fold paralysis[199, 221]. In patients with SD, abnormal (increased/decreased) brain activity in the brain regions typically active during normal phonation may be related to voice symptoms [193, 214, 216, 217]. Although Ludlow et al[37] have suggested that MTD patients, unlike SD patients, [37] do not have neurologic motor control disorders, the neurophysiological mechanism of laryngeal hyperfunction and postural control leading to voice pitch limitation in patients with MTD remains unexplored.

## 5. Objectives

The purpose of the study was threefold: (1) to determine the vocal characteristics in patients treated with VNS in comparison with healthy controls; (2) to investigate brain activity during phonation in women with MTD in comparison with healthy controls using a specific fMRI protocol; and (3) to detect brain activity during phonation in healthy female singers with normal vocal characteristics and supraglottic compression using the specific fMRI protocol.

The following research questions are addressed:

### Purpose 1

Does VNS influence vocal characteristics? (Chapter 2)

The study of the impact of VNS on objective and subjective vocal characteristics enables a unique possibility to assess alteration of vocal characteristics that has developed as a result of stimulating the SLN and/or the RLN and thereby exciting either the afferent and/or efferent nerve fibers of the laryngeal system. We hypothesized that, compared with healthy controls, patients treated with VNS may have a significant decrease in their objective vocal quality (decreased DSI value) together with disordered perceptual vocal characteristics, and an ensuing impact of the voice disorder on their quality of life (QOL). Moreover, a significant correlation was hypothesized between the amount of stimulation and the presence of disturbed acoustic parameters.

## Purpose 2

Is brain activity during phonation in healthy people detected with the specific fMRI protocol? (Chapter 3)

CNS control of voice with emphasis on phonation has previously been poorly investigated. Our study has proposed a blocked design[1] fMRI experiment to investigate the CNS control of voice with emphasis on laryngeal control of phonation. We hypothesized that a primary region activated in association with vocal pitch (comfortable and high) adaptation/modulation, where both auditory and somatosensory inputs were different, would be the auditory cortex, more specifically the STG[65, 66, 75, 77, 144-146] and that this would be observed by using the proposed fMRI protocol.

Is altered brain activity during phonation in women with MTD detected with the specific fMRI protocol? (Chapter 4)

Neuroimaging studies of voice disorders have previously been limited to a few specific voice pathologies such as SD [193, 214-217], Parkinson's disease [218-220], and idiopathic unilateral vocal fold paralysis [199, 221]. No previous studies have evaluated neural correlates of phonation in MTD. In this study we investigated brain activity during phonation in women with MTD in comparison with healthy controls in order to determine altered brain activities of phonation control in patients with MTD. We hypothesized that, compared with healthy controls, MTD patients may have altered brain activities related to phonation control. Moreover, it was hypothesized that the mechanism of vocal learning and adaptation[184] explains vocal hyperfunction during phonation in MTD patients.

---

[1] In a blocked design, a condition is presented continuously for an extended time interval (block) to maintain cognitive engagement, and different task conditions are usually alternating in time.

## Purpose 3

Is brain activity during phonation in healthy female singers with supraglottic compression detected with the specific fMRI protocol? (Chapter 5)

Laryngeal hyperfunction is a sign of abuse or misuse of the vocal mechanism which is commonly reported in patients with functional voice disorders. However, recent studies have demonstrated that laryngeal hyperfunction may be present in normal speaking and singing. However, there are no studies that evaluate the neurophysiological mechanism of supraglottic compression during phonation in healthy people. In this study we have investigated brain activity during phonation in healthy female singers without voice disorders and with supraglottic compression using the proposed protocol. We hypothesized that the proposed fMRI protocol would detect brain activation during phonation and reveal the neural mechanism that may affect laryngeal supraglotic compression during phonation in healthy female singers. The results of this study also contribute to the necessary refinement of the fMRI protocol.

This PhD thesis comprises a collection of journal papers published in the course of this PhD period (Chapter 2 – 5).

## 5. References


**1.** Van Houtte E, Van Lierde K, D'haeseleer E, Claeys S. The prevalence of laryngeal pathology in a treatment□seeking population with dysphonia. *The Laryngoscope.* 2010;120:306-312.

**2.** Van Lierde K, Claeys S, De Bodt M, Van Cauwenberge P. Long-term outcome of hyperfunctional voice disorders based on a multiparameter approach. *Journal of Voice.* 2007;21:179-188.

**3.** Boone DR, McFarlane SC, Von Berg SL, Zraick RI. The voice and voice therapy. 2005.

**4.** Stemple JC, Glaze LE, Gerdeman BK. *Clinical voice pathology: Theory and management*: Cengage Learning; 2000.

**5.** Mayerhoff RM, Guzman M, Jackson□Menaldi C, et al. Analysis of supraglottic activity during vocalization in healthy singers. *The Laryngoscope.* 2014;124:504-509.

**6.** Guzman M, Ortega A, Olavarria C, et al. Comparison of supraglottic activity and spectral slope between theater actors and vocally untrained subjects. *Journal of Voice.* 2015.

**7.** Stager SV, Bielamowicz SA, Regnell JR, Gupta A, Barkmeier JM. Supraglottic ActivityEvidence of Vocal Hyperfunction or Laryngeal Articulation? *Journal of speech, language, and hearing research.* 2000;43:229-238.

**8.** Behrman A, Dahl LD, Abramson AL, Schutte HK. Anterior-Posterior and medial compression of the supraglottis: signs of nonorganic dysphoniaor normal postures? *Journal of Voice.* 2003;17:403-410.

**9.** Stager SV, Bielamowicz S, Gupta A, Marullo S, Regnell JR, Barkmeier J. Quantification of static and dynamic supraglottic activity. *Journal of Speech, Language, and Hearing Research.* 2001;44:1245-1256.

**10.** Sama A, Carding P, Price S, Kelly P, Wilson J. The clinical features of functional dysphonia. *The Laryngoscope.* 2001;111:458-463.

**11.** Lawrence V. Laryngological observations on belting. *J Res Singing.* 1979;2:26-28.

**12.** Yanagisawa E, Estill J, Kmucha ST, Leder SB. The contribution of aryepiglottic constriction to "ringing" voice quality—a videolaryngoscopic study with acoustic analysis. *Journal of Voice.* 1989;3:342-350.

**13.** Pershall KE, Boone DR. Supraglottic contribution to voice quality. *Journal of Voice.* 1987;1:186-190.

**14.** Hsieh T, Chen M, McAfee A, Kifle Y. Sleep-related breathing disorder in children with vagal nerve stimulators. *Pediatric neurology.* 2008;38:99-103.

**15.** Charous SJ, Kempster G, Manders E, Ristanovic R. The effect of vagal nerve stimulation on voice. *The Laryngoscope.* 2001;111:2028-2031.

**16.** Roy N. Functional dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2003;11:144-148.

**17.** Dworkin JP, Meleca RJ, Abkarian G. Muscle tension dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2000;8:169-173.

**18.** Van Houtte E, Claeys S, D'haeseleer E, Wuyts F, Van Lierde K. An examination of surface EMG for the assessment of muscle tension dysphonia. *Journal of Voice.* 2013;27:177-186.

**19.** Van Houtte E, Van Lierde K, D'haeseleer E, Van Imschoot B, Claeys S. UES Pressure During Phonation Using High-resolution Manometry and 24-h Dual-probe pH-metry in Patients with Muscle Tension Dysphonia. *Dysphagia.* 2012;27:198-209.

**20.** Van Houtte E, Van Lierde K, Claeys S. Pathophysiology and treatment of muscle tension dysphonia: a review of the current knowledge. *Journal of Voice.* 2011;25:202-207.

**21.** Simonyan K, Horwitz B, Jarvis ED. Dopamine regulation of human speech and bird song: a critical review. *Brain and language.* 2012;122:142-150.

**22.** Wyke B. Reflexogenic contributions to vocal fold control systems. In: Titze IR, Ronald CS, eds. *Vocal fold physiology: biomechanics, acoustics and phonatory control* The Denver Center for the Performing Arts, Inc., Denver, Colorado: College-Hill; 1983:138-141.

**23.** Ludlow CL. Laryngeal Reflexes: Physiology, Technique, and Clinical Use. *Journal of Clinical Neurophysiology.* 2015;32:284-293.

**24.** Newman JD. *The physiological control of mammalian vocalization*: Springer Science & Business Media; 2012.

**25.** Ludlow CL. Central Nervous System Control of Voice and Swallowing. *Journal of Clinical Neurophysiology.* 2015;32:294-303.

**26.** Kelchner LN, Brehm SB, Weinrich B. *Pediatric Voice: A Modern, Collaborative Approach to Care*: Plural Publishing; 2014.

**27.** Association AS-L-H. Definitions of communication disorders and variations. 1993.

**28.** Ben-Menachem E. Vagus-nerve stimulation for the treatment of epilepsy. *The Lancet Neurology.* 2002;1:477-482.

**29.** Shaffer MJ, Szabo CA, Jackson CE, Simpson CB. Vagal nerve stimulation: clinical and electrophysiological effects on vocal fold function. *Annals of Otology, Rhinology & Laryngology.* 2005;114:7-14.

**30.** Morrison M, Rammage L. Muscle misuse voice disorders: description and classification. *Acta oto-laryngologica.* 1993;113:428-434.

**31.** Morrison M, Rammage L, Belisle G, Pullan C, Nichol H. Muscular tension dysphonia. *The Journal of otolaryngology.* 1983;12:302-306.

**32.** Roy N, Ford CN, Bless DM. Muscle tension dysphonia and spasmodic dysphonia: the role of manual laryngeal tension reduction in diagnosis and management. *Annals of Otology, Rhinology & Laryngology.* 1996;105:851-856.

**33.** Angsuwarangsee T, Morrison M. Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice.* 2002;16:333-343.

**34.** Kooijman P, De Jong F, Oudes M, Huinck W, Van Acht H, Graamans K. Muscular tension and body posture in relation to voice handicap and voice quality in teachers with persistent voice complaints. *Folia phoniatrica et logopaedica.* 2005;57:134-147.

**35.** Lowell SY, Kelley RT, Colton RH, Smith PB, Portnoy JE. Position of the hyoid and larynx in people with muscle tension dysphonia. *The Laryngoscope.* 2012;122:370-377.

**36.** Khoddami SM, Nakhostin Ansari N, Izadi F, Talebian Moghadam S. The assessment methods of laryngeal muscle activity in muscle tension dysphonia: a review. *The Scientific World Journal.* 2013;2013.

**37.** Ludlow CL, Adler CH, Berke GS, et al. Research priorities in spasmodic dysphonia. *Otolaryngology-Head and Neck Surgery.* 2008;139:495-505. e491.

**38.** Johns MM. Update on the etiology, diagnosis, and treatment of vocal fold nodules, polyps, and cysts. *Current opinion in otolaryngology & head and neck surgery.* 2003;11:456-461.

**39.** Koufman J, Blalock P. Functional voice disorders. *Otolaryngologic Clinics of North America.* 1991;24:1059-1073.

**40.** Roy N, Bless DM. Personality Traits and Psychological Factors in Voice PathologyA Foundation for Future Research. *Journal of Speech, Language, and Hearing Research.* 2000;43:737-748.

**41.** Roy N, Bless DM, Heisey D. Personality and voice disorders: a multitrait-multidisorder analysis. *Journal of Voice.* 2000;14:521-548.

**42.** Von Helmholtz H. *Handbuch der physiologischen Optik*: Voss; 1867.

**43.** Hocevar-Boltezar I, Janko M, Zargi M. Role of surface EMG in diagnostics and treatment of muscle tension dysphonia. *Acta Otolaryngologica.* 1998;118:739-743.

**44.** Redenbaugh MA, Reich AR. Surface EMG and related measures in normal and vocally hyperfunctional speakers. *Journal of Speech and Hearing Disorders.* 1989;54:68-73.

**45.** Stepp CE, Heaton JT, Braden MN, Jette ME, Stadelman-Cohen TK, Hillman RE. Comparison of neck tension palpation rating systems with surface electromyographic and acoustic measures in vocal hyperfunction. *Journal of Voice.* 2011;25:67-75.

**46.** Stepp CE, Heaton JT, Jetté ME, Burns JA, Hillman RE. Neck surface electromyography as a measure of vocal hyperfunction before and after injection laryngoplasty. *The Annals of otology, rhinology, and laryngology.* 2010;119:594.

**47.** Stepp CE, Heaton JT, Stadelman-Cohen TK, Braden MN, Jetté ME, Hillman RE. Characteristics of phonatory function in singers and nonsingers with vocal fold nodules. *Journal of voice.* 2011;25:714-724.

**48.** Stepp CE, Hillman RE, Heaton JT. Use of neck strap muscle intermuscular coherence as an indicator of vocal hyperfunction. *Neural Systems and Rehabilitation Engineering, IEEE Transactions on.* 2010;18:329-335.

**49.** Bove M, Ruth M, Cange L, Månsson I. 24-H pharyngeal pH monitoring in healthy volunteers: a normative study. *Scandinavian journal of gastroenterology.* 2000;35:234-241.

**50.** Jacobson E. Progressive relaxation. 1938.

**51.** Froeschels E. Chewing method as therapy: A discussion with some philosophical conclusions. *AMA archives of otolaryngology.* 1952;56:427-434.

**52.** Boone DR, McFarlane SC. A critical view of the yawn-sigh as a voice therapy technique. *Journal of Voice.* 1993;7:75-80.

**53.** Stemple JC, Weiler E, Whitehead W, Komray R. Electromyographic biofeedback training with patients exhibiting a hyperfunctional voice disorder. *The Laryngoscope.* 1980;90:471-476.

**54.** Roy N, Bless DM, Heisey D, Ford CN. Manual circumlaryngeal therapy for functionaldysphonia: An evaluation of short-and long-term treatment outcomes. *Journal of Voice.* 1997;11:321-331.

**55.** Van Houtte E, Claeys S, Wuyts F, Van Lierde K. The impact of voice disorders among teachers: vocal complaints, treatment-seeking behavior, knowledge of vocal care, and voice-related absenteeism. *Journal of voice.* 2011;25:570-575.

**56.** Carding PN, Horsley IA. An evaluation study of voice therapy in non□organic dysphonia. *International Journal of Language & Communication Disorders.* 1992;27:137-158.

**57.** Speyer R. Effects of voice therapy: a systematic review. *Journal of Voice.* 2008;22:565-580.

**58.** Lundy DS, Casiano RR, Landy HJ, Gallo J, Gallo B, Ramsey RE. Effects of vagal nerve stimulation on laryngeal function. *Journal of Voice.* 1993;7:359-364.

**59.** Bernards CM. An unusual cause of airway obstruction during general anesthesia with a laryngeal mask airway. *The Journal of the American Society of Anesthesiologists.* 2004;100:1017-1018.

**60.** Schachter SC, Saper CB. Vagus nerve stimulation. *Epilepsia.* 1998;39:677-686.

**61.** Morrison M, Rammage L, Emami A. The irritable larynx syndrome. *Journal of Voice.* 1999;13:447-455.

**62.** Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cerebral Cortex.* 2008;18:837-845.

**63.** Turkeltaub PE, Eden GF, Jones KM, Zeffiro TA. Meta-analysis of the functional neuroanatomy of single-word reading: method and validation. *Neuroimage.* 2002;16:765-780.

**64.** Bohland JW, Guenther FH. An fMRI investigation of syllable sequence production. *Neuroimage.* 2006;32:821-841.

**65.** Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. *Neuroimage.* 2008;39:1429-1443.

**66.** Guenther FH, Ghosh SS, Tourville JA. Neural modeling and imaging of the cortical interactions underlying syllable production. *Brain and language.* 2006;96:280-301.

**67.** Guenther FH, Hampson M, Johnson D. A theoretical investigation of reference frames for the planning of speech movements. *Psychological review.* 1998;105:611.

**68.** Tourville JA, Guenther FH. The DIVA model: A neural theory of speech acquisition and production. *Language and cognitive processes.* 2011;26:952-981.

**69.** Guenther FH. Cortical interactions underlying the production of speech sounds. *Journal of communication disorders.* 2006;39:350-365.

**70.** Simonyan K, Horwitz B. Laryngeal motor cortex and control of speech in humans. *The Neuroscientist.* 2011;17 (2):197–208.

**71.** Zarate JM. The neural control of singing. *Frontiers in human neuroscience.* 2013;7.

**72.** Civier O, Tasko SM, Guenther FH. Overreliance on auditory feedback may lead to sound/syllable repetitions: simulations of stuttering and fluency-inducing conditions with a neural model of speech production. *Journal of fluency disorders.* 2010;35:246-279.

**73.** Flagmeier SG, Ray KL, Parkinson AL, et al. The neural changes in connectivity of the voice network during voice pitch perturbation. *Brain and language.* 2014;132:7-13.

**74.** Hickok G. Computational neuroanatomy of speech production. *Nature Reviews Neuroscience.* 2012;13:135-145.

**75.** Hickok G, Houde J, Rong F. Sensorimotor integration in speech processing: computational basis and neural organization. *Neuron.* 2011;69:407-422.

**76.** Jones JA, Munhall K. Remapping auditory-motor representations in voice production. *Current Biology.* 2005;15:1768-1772.

**77.** Parkinson AL, Flagmeier SG, Manes JL, Larson CR, Rogers B, Robin DA. Understanding the neural mechanisms involved in sensory control of voice production. *Neuroimage.* 2012;61:314-322.

**78.** Scheerer NE, Jones JA. The predictability of frequency□altered auditory feedback changes the weighting of feedback and feedforward input for speech motor control. *European Journal of Neuroscience.* 2014;40:3793-3806.

**79.** Ackermann H. Cerebellar contributions to speech production and speech perception: psycholinguistic and neurobiological perspectives. *Trends in neurosciences.* 2008;31:265-272.

**80.** Ackermann H, Hage SR, Ziegler W. Brain mechanisms of acoustic communication in humans and nonhuman primates: An evolutionary perspective. *Behavioral and Brain Sciences.* 2014;37:529-546.

**81.** Penfield W, Roberts L. *Speech and brain mechanisms*: Princeton University Press; 2014.

**82.** Simonyan K. The laryngeal motor cortex: its organization and connectivity. *Current opinion in neurobiology.* 2014;28:15-21.

**83.** Jürgens U. Neural pathways underlying vocal control. *Neuroscience & Biobehavioral Reviews.* 2002;26:235-258.

**84.** Jürgens U. A study of the central control of vocalization using the squirrel monkey. *Medical engineering & physics.* 2002;24:473-477.

**85.** Schmidt RF, Wiesendanger M. Motorische Systeme. *Physiologie des Menschen*: Springer; 1987:87-131.

**86.** Penfield W, Rasmussen T. *The cerebral cortex of man; a clinical study of localization of function*. New York: Macmillan Publishers Ltd; 1950.

**87.** Rödel RM, Olthoff A, Tergau F, et al. Human cortical motor representation of the larynx as assessed by transcranial magnetic stimulation (TMS). *The Laryngoscope.* 2004;114:918-922.

**88.** Loucks TM, Poletto CJ, Simonyan K, Reynolds CL, Ludlow CL. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. *Neuroimage.* 2007;36:131-143.

**89.** Kuypers H. Corticobulbar connexions to the pons and lower brain-stem in man. *Brain.* 1958;81:364-388.

**90.** Schulz G, Varga M, Jeffires K, Ludlow C, Braun A. Functional neuroanatomy of human vocalization: an H215O PET study. *Cerebral cortex.* 2005;15:1835-1847.

**91.** Chang S-E, Kenney MK, Loucks TM, Poletto CJ, Ludlow CL. Common neural substrates support speech and non-speech vocal tract gestures. *Neuroimage.* 2009;47:314-325.

**92.** Simonyan K, Ostuni J, Ludlow CL, Horwitz B. Functional but not structural networks of the human laryngeal motor cortex show left hemispheric lateralization during syllable but not breathing production. *The Journal of Neuroscience.* 2009;29:14912-14923.

**93.** Scharff C. Neurobiology of human language and its evolution: Primate and Nonprimate Perspectives. *Frontiers in Evolutionary Neuroscience* 2013;5:1.

**94.** Ramsay S, Adams L, Murphy K, et al. Regional cerebral blood flow during volitional expiration in man: a comparison with volitional inspiration. *The Journal of physiology.* 1993;461:85-101.

**95.** Laukka P, Åhs F, Furmark T, Fredrikson M. Neurofunctional correlates of expressed vocal affect in social phobia. *Cognitive, Affective, & Behavioral Neuroscience.* 2011;11:413-425.

**96.** Jürgens U. The neural control of vocalization in mammals: a review. *Journal of Voice.* 2009;23:1-10.

**97.** Hage SR. Localization of the central pattern generator for vocalization. In: Brudzynski SM, ed. *Handbook of mammalian vocalization: An integrative neuroscience approach.* Vol 19. Amsterdam Boston; Heidelberg; London; New York; Oxford; Paris; San Diego; San Francisco; Singapore; Sydney; Tokyo: Academic Press is an imprint of Elsevier; 2010:329-337.

**98.** Hage SR. Neuronal networks involved in the generation of vocalization. In: Brudzynski SM, ed. *Handbook of mammalian vocalization: An integrative neuroscience approach.* Vol 19. Amsterdam Boston; Heidelberg; London; New York; Oxford; Paris; San Diego; San Francisco; Singapore; Sydney; Tokyo: Academic Press is an imprint of Elsevier; 2010:339-349.

**99.** Thoms G, Jürgens U. Common input of the cranial motor nuclei involved in phonation in squirrel monkey. *Experimental neurology.* 1987;95:85-99.

**100.** Bass AH. Central pattern generator for vocalization: is there a vertebrate morphotype? *Current opinion in neurobiology.* 2014;28:94-100.

**101.** Hannig S, Jürgens U. Projections of the ventrolateral pontine vocalization area in the squirrel monkey. *Experimental brain research.* 2006;169:92-105.

**102.** Iwatsubo T, Kuzuhara S, Kanemitsu A, Shimada H, Toyokura Y. Corticofugal projections to the motor nuclei of the brainstem and spinal cord in humans. *Neurology.* 1990;40:309-309.

**103.** Simonyan K, Ludlow CL, Vortmeyer AO. Brainstem pathology in spasmodic dysphonia. *The Laryngoscope.* 2010;120:121-124.

**104.** Aziz-Zadeh L, Sheng T, Gheytanchi A. Common premotor regions for the perception and production of prosody and correlations with empathy and prosodic ability. *PLoS One.* 2010;5:1-8.

**105.** Wattendorf E, Westermann B, Fiedler K, Kaza E, Lotze M, Celio MR. Exploration of the neural correlates of ticklish laughter by functional magnetic resonance imaging. *Cerebral Cortex.* 2013;23:1280-1289.

**106.** Pichon S, Kell CA. Affective and sensorimotor components of emotional prosody generation. *The Journal of Neuroscience.* 2013;33:1640-1650.

**107.** Ambalavanar R, Tanaka Y, Damirjian M, Ludlow CL. Laryngeal afferent stimulation enhances Fos immunoreactivity in periaqueductal gray in the cat. *Journal of Comparative Neurology.* 1999;409:411-423.

**108.** Jürgens U, Kirzinger A. The laryngeal sensory pathway and its role in phonation. A brain lesioning study in the squirrel monkey. *Experimental brain research.* 1985;59:118-124.

**109.** Shiba K, Miura T, Yuza J, Sakamoto T, Nakajima Y. Laryngeal afferent inputs during vocalization in the cat. *Neuroreport.* 1999;10:987-991.

**110.** Yoshida Y, Tanaka Y, Hirano M, Nakashima T. Sensory innervation of the pharynx and larynx. *The American journal of medicine.* 2000;108:51-61.

**111.** Barkmeier JM, Bielamowicz S, Takeda N, Ludlow CL. Modulation of laryngeal responses to superior laryngeal nerve stimulation by volitional swallowing in awake humans. *Journal of neurophysiology.* 2000;83:1264-1272.

**112.** Gacek RR. Localization of laryngeal motor neurons in the kitten. *The Laryngoscope.* 1975;85:1841-1859.

**113.** Mrini A, Jean A. Synaptic organization of the interstitial subdivision of the nucleus tractus solitarii and of its laryngeal afferents in the rat. *Journal of Comparative Neurology.* 1995;355:221-236.

**114.** Tanaka Y, Yoshida Y, Hirano M, Morimoto M, Kanaseki T. Distribution of sensory nerve fibers in the larynx and pharynx: an HRP study in cats. In: Hirano M, Kirchner J, Bless D, eds. *Neurolaryngology: Recent Advances,* . Boston, MA: College-Hill; 1987:27-45.

**115.** Yoshida Y, Miyazaki T, Hirano M, Shin T, Kanaseki T. Arrangement of Motoneurons Innervating the Intrinsic Laryn-Geal Muscles of Cats as Demonstrated by Horseradish Peroxidas. *Acta oto-laryngologica.* 1982;94:329-334.

**116.** Bauer JJ, Mittal J, Larson CR, Hain TC. Vocal responses to unanticipated perturbations in voice loudness feedback: An automatic mechanism for stabilizing voice amplitude. *The Journal of the Acoustical Society of America.* 2006;119:2363-2371.

**117.** Brumm H, Zollinger SA. The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour.* 2011;148:1173-1198.

**118.** Lombard E. Le signe de l'elevation de la voix. *Ann. Maladies Oreille, Larynx, Nez, Pharynx.* 1911;37:25.

**119.** Borg E, Counter S, Rösler G. Theories of middle-ear muscle function. In: Silman S, ed. *The acoustic reflex: Basic principles and clinical applications*. New York, NY: Academic Press; 1984:63-99.

**120.** Goldberg R, Henson O. Changes in cochlear mechanics during vocalization: evidence for a phasic medial efferent effect. *Hearing research.* 1998;122:71-81.

**121.** Hage SR, Jürgens U, Ehret G. Audio–vocal interaction in the pontine brainstem during self□ initiated vocalization in the squirrel monkey. *European Journal of Neuroscience.* 2006;23:3297-3308.

**122.** Perera L, Kern M, Hofmann C, et al. Manometric evidence for a phonation-induced UES contractile reflex. *American Journal of Physiology-Gastrointestinal and Liver Physiology.* 2008;294:G885-G891.

**123.** Jones JA, Munhall K. The role of auditory feedback during phonation: studies of Mandarin tone production. *Journal of Phonetics.* 2002;30:303-320.

**124.** Houde JF, Niziolek C, Kort N, Agnew Z, Nagarajan SS. Simulating a state feedback model of speaking. *10th International Seminar on Speech Production*2014:202-205.

**125.** Parkinson AL, Behroozmand R, Ibrahim N, Korzyukov O, Larson CR, Robin DA. Effective connectivity associated with auditory error detection in musicians with absolute pitch. *Frontiers in neuroscience.* 2014;8.

**126.** Cramer SC, Sur M, Dobkin BH, et al. Harnessing neuroplasticity for clinical applications. *Brain.* 2011;134:1591-1609.

**127.** Ludlow CL, Hoit J, Kent R, et al. Translating principles of neural plasticity into research on speech motor control recovery and rehabilitation. *Journal of Speech, Language, and Hearing Research.* 2008;51:S240-S258.

**128.** Nudo RJ. Plasticity. *NeuroRx.* 2006;3:420-427.

**129.** Levin MF, Kleim JA, Wolf SL. What do motor "recovery" and "compensation" mean in patients following stroke? *Neurorehabilitation and neural repair.* 2008.

**130.** Brosh I, Barkai E. Learning-induced long-term synaptic modifications in the olfactory cortex. *Current neurovascular research.* 2004;1:389-395.

**131.** Herbst CT, Hess M, Müller F, Švec JG, Sundberg J. Glottal adduction and subglottal pressure in singing. *Journal of Voice.* 2015;29:391-402.

**132.** Tinazzi M, Zanette G, Volpato D, et al. Neurophysiological evidence of neuroplasticity at multiple levels of the somatosensory system in patients with carpal tunnel syndrome. *Brain.* 1998;121:1785-1794.

**133.** Urasaki E, Genmoto T, Wada S-i, Yokota A, Akamatsu N. Dynamic changes in area 1 somatosensory cortex during transient sensory deprivation: A preliminary study. *Journal of clinical neurophysiology.* 2002;19:219-231.

**134.** Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *The Journal of neuroscience.* 1998;18:7000-7007.

**135.** Bellemare F, Woods J, Johansson R, Bigland-Ritchie B. Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *Journal of Neurophysiology.* 1983;50:1380-1392.

**136.** Larson CR, Sun J, Hain TC. Effects of simultaneous perturbations of voice pitch and loudness feedback on voice F0 and amplitude control. *The Journal of the Acoustical Society of America.* 2007;121:2862-2872.

**137.** Jones JA, Munhall KG. Perceptual calibration of F0 production: evidence from feedback perturbation. *The Journal of the Acoustical Society of America.* 2000;108:1246-1251.

**138.** Pynn LK, DeSouza JF. The function of efference copy signals: implications for symptoms of schizophrenia. *Vision research.* 2013;76:124-133.

**139.** Golfinopoulos E, Tourville JA, Guenther FH. The integration of large-scale neural network modeling and functional brain imaging in speech motor control. *Neuroimage.* 2010;52:862-874.

**140.** Shadmehr R, Krakauer JW. A computational neuroanatomy for motor control. *Experimental Brain Research.* 2008;185:359-381.

**141.** Hickok G. The functional neuroanatomy of language. *Physics of life reviews.* 2009;6:121-143.

**142.** Hickok G, Poeppel D. The cortical organization of speech processing. *Nature Reviews Neuroscience.* 2007;8:393-402.

**143.** Durisko C, Fiez JA. Functional activation in the cerebellum during working memory and simple speech tasks. *Cortex.* 2010;46:896-906.

**144.** Behroozmand R, Larson CR. Error-dependent modulation of speech-induced auditory suppression for pitch-shifted voice feedback. *BMC neuroscience.* 2011;12:54.

**145.** Zarate JM, Zatorre RJ. Experience-dependent neural substrates involved in vocal pitch regulation during singing. *Neuroimage.* 2008;40:1871-1887.

**146.** Zarate JM, Zatorre RJ. Neural substrates governing audiovocal integration for vocal pitch regulation in singing. *Annals of the New York Academy of Sciences.* 2005;1060:404-408.

**147.** Parkinson AL, Korzyukov O, Larson CR, Litvak V, Robin DA. Modulation of effective connectivity during vocalization with perturbed auditory feedback. *Neuropsychologia.* 2013;51:1471-1480.

**148.** Heinks-Maldonado TH, Houde JF. Compensatory responses to brief perturbations of speech amplitude. *Acoustics Research Letters Online.* 2005;6:131-137.

**149.** Houde JF, Jordan MI. Sensorimotor Adaptation of Speech ICompensation and Adaptation. *Journal of Speech, Language, and Hearing Research.* 2002;45:295-310.

**150.** Porter BA, Khodaparast N, Fayyaz T, et al. Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. *Cerebral Cortex.* 2011;22:2365-2374.

**151.** Park K-H, Cho S-H, Song C-E, Kim D-H, Kim H-T. Neuroimmunological activation of the afferent laryngeal neural circuit in experimentally induced laryngeal inflammation. *Acta oto-laryngologica.* 2005;125:184-190.

**152.** Simonyan K, Feng X, Henriquez VM, Ludlow CL. Combined laryngeal inflammation and trauma mediate long-lasting immunoreactivity response in the brainstem sensory nuclei in the rat. *Frontiers in integrative neuroscience.* 2012;6.

**153.** Düsterhöft F, Häusler U, Jürgens U. On the search for the vocal pattern generator. A single□unit recording study. *Neuroreport.* 2000;11:2031-2034.

**154.** Morrison M, Rammage L. The irritable larynx syndrome as a central sensitivity syndrome. *Canadian Journal of Speech-Language-Pathology and Audiology* 2010;34(4):282-289.

**155.** Hays SA, Rennaker RL, Kilgard MP. Targeting plasticity with vagus nerve stimulation to treat neurological disease. *Progress in brain research.* 2013;207:275.

**156.** Narayanan JT, Watts R, Haddad N, Labar DR, Li PM, Filippi CG. Cerebral activation during vagus nerve stimulation: a functional MR study. *Epilepsia.* 2002;43:1509-1514.

**157.** Van Laere K, Vonck K, Boon P, Brans B, Vandekerckhove T, Dierckx R. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. *Journal of Nuclear Medicine.* 2000;41:1145-1154.

**158.** Van Laere K, Vonck K, Boon P, Versijpt J, Dierckx R. Perfusion SPECT changes after acute and chronic vagus nerve stimulation in relation to prestimulus condition and long-term clinical efficacy. *Journal of Nuclear Medicine.* 2002;43:733-744.

**159.** Yakunina N, Kim SS, Nam EC. Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation: Technology at the Neural Interface.* 2017;20:290-300.

**160.** Bohning DE, Lomarev MP, Denslow S, Nahas Z, Shastri A, George MS. Feasibility of vagus nerve stimulation–synchronized blood oxygenation level–dependent functional MRI. *Investigative Radiology.* 2001;36:470-479.

**161.** Ring H, White S, Costa D, et al. A SPECT study of the effect of vagal nerve stimulation on thalamic activity in patients with epilepsy. *Seizure.* 2000;9:380-384.

**162.** Vonck K, Boon P, Laere K, et al. Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. *Epilepsia.* 2000;41:601-609.

**163.** Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. *Seizure.* 2008;17:699-706.

**164.** Vonck K, Van Laere K, Dedeurwaerdere S, Caemaert J, De Reuck J, Boon P. The mechanism of action of vagus nerve stimulation for refractory epilepsy: the current status. *Journal of clinical neurophysiology.* 2001;18:394-401.

**165.** Lomarev M, Denslow S, Nahas Z, Chae J-H, George MS, Bohning DE. Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects. *Journal of psychiatric research.* 2002;36:219-227.

**166.** Liu WC, Mosier K, Kalnin A, Marks D. BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. *Journal of Neurology, Neurosurgery & Psychiatry.* 2003;74:811-813.

**167.** Nahas Z, Teneback C, Jeong-Ho C, et al. Serial vagus nerve stimulation functional MRI in treatment-resistant depression. *Neuropsychopharmacology.* 2007;32:1649.

**168.** Dietrich S, Smith J, Scherzinger C, et al. A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI/Funktionelle Magnetresonanztomographie zeigt Aktivierungen des Hirnstamms und weiterer zerebraler Strukturen unter transkutaner Vagusnervstimulation. *Biomedizinische Technik/Biomedical Engineering.* 2008;53:104-111.

**169.** Kraus T, Hösl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of neural transmission.* 2007;114:1485-1493.

**170.** Kraus T, Kiess O, Hösl K, Terekhin P, Kornhuber J, Forster C. CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal–a pilot study. *Brain stimulation.* 2013;6:798-804.

**171.** Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain stimulation.* 2015;8:624-636.

**172.** Frangos E, Komisaruk BR. Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. *Brain stimulation.* 2017;10:19-27.

**173.** Frangos E, Richards EA, Bushnell MC. Do the psychological effects of vagus nerve stimulation partially mediate vagal pain modulation? *Neurobiology of Pain.* 2017.

**174.** Picard N, Strick PL. Motor areas of the medial wall: a review of their location and functional activation. *Cerebral cortex.* 1996;6:342-353.

**175.** Ludlow CL. Central nervous system control of the laryngeal muscles in humans. *Respiratory physiology & neurobiology.* 2005;147:205-222.

**176.** Ludlow CL. Central nervous system control of interactions between vocalization and respiration in mammals. *Head & neck.* 2011;33:S21-S25.

**177.** Hammer MJ, Barlow SM. Laryngeal somatosensory deficits in Parkinson's disease: implications for speech respiratory and phonatory control. *Experimental brain research.* 2010;201:401-409.

**178.** Chen Z, Liu P, Jones JA, Huang D, Liu H. Sex-related differences in vocal responses to pitch feedback perturbations during sustained vocalization. *The Journal of the Acoustical Society of America.* 2010;128:EL355-EL360.

**179.** Shiozawa P, Silva MEd, Carvalho TCd, Cordeiro Q, Brunoni AR, Fregni F. Transcutaneous vagus and trigeminal nerve stimulation for neuropsychiatric disorders: a systematic review. *Arquivos de neuro-psiquiatria.* 2014;72:542-547.

**180.** Mohr P, Rodriguez M, Slavíčková A, Hanka J. The application of vagus nerve stimulation and deep brain stimulation in depression. *Neuropsychobiology.* 2011;64:170-181.

**181.** Brunoni AR, Teng CT, Correa C, et al. Neuromodulation approaches for the treatment of major depression: challenges and recommendations from a working group meeting. *Arquivos de neuro-psiquiatria.* 2010;68:433-451.

**182.** Schlaepfer TE, George MS. WFSBP guidelines on brain stimulation treatments in psychiatry. *The World Journal of Biological Psychiatry.* 2010;11:2-18.

**183.** Fanselow EE, Reid AP, Nicolelis MA. Reduction of pentylenetetrazole-induced seizure activity in awake rats by seizure-triggered trigeminal nerve stimulation. *The Journal of Neuroscience.* 2000;20:8160-8168.

**184.** Urberg-Carlson KE. *Muscle tension dysphonia as a disorder of motor learning*, University of Minnesota; 2013.

**185.** Urberg□Carlson K, Munson B, Watson P. Adaptive learning response to auditory perturbation of voice quality feedback. *The Journal of the Acoustical Society of America.* 2011;129:2659-2659.

**186.** Callan DE, Kent RD, Guenther FH, Vorperian HK. An auditory-feedback-based neural network model of speech production that is robust to developmental changes in the size and shape of the articulatory system. *Journal of speech, language, and hearing research.* 2000;43:721-736.

**187.** Shiller DM, Sato M, Gracco VL, Baum SR. Perceptual recalibration of speech sounds following speech motor learning. *The Journal of the Acoustical Society of America.* 2009;125:1103-1113.

**188.** Nasir SM, Ostry DJ. Auditory plasticity and speech motor learning. *Proceedings of the National Academy of Sciences.* 2009;106:20470-20475.

**189.** Altman KW, Atkinson C, Lazarus C. Current and emerging concepts in muscle tension dysphonia: a 30-month review. *Journal of Voice.* 2005;19:261-267.

**190.** Morrison M, Nichol H, Rammage L. Diagnostic criteria in functional dysphonia. *The Laryngoscope.* 1986;96:1-8.

**191.** Rubin AD, Praneetvatakul V, Gherson S, Moyer CA, Sataloff RT. Laryngeal hyperfunction during whispering: reality or myth? *Journal of Voice.* 2006;20:121-127.

**192.** Huang J, Carr TH, Cao Y. Comparing cortical activations for silent and overt speech using event□related fMRI. *Human brain mapping.* 2002;15:39-53.

**193.** Haslinger B, Erhard P, Dresel C, Castrop F, Roettinger M, Ceballos-Baumann A. "Silent event-related" fMRI reveals reduced sensorimotor activation in laryngeal dystonia. *Neurology.* 2005;65:1562-1569.

**194.** Özdemir E, Norton A, Schlaug G. Shared and distinct neural correlates of singing and speaking. *Neuroimage.* 2006;33:628-635.

**195.** Murphy K, Corfield D, Guz A, et al. Cerebral areas associated with motor control of speech in humans. *Journal of Applied Physiology.* 1997;83:1438-1447.

**196.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2016.

**197.** Brown S, Laird AR, Pfordresher PQ, Thelen SM, Turkeltaub P, Liotti M. The somatotopy of speech: phonation and articulation in the human motor cortex. *Brain and cognition.* 2009;70:31-41.

**198.** Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage.* 2006;31:1453-1474.

**199.** Galgano JF, Peck KK, Branski RC, et al. Correlation between functional MRI and voice improvement following type I thyroplasty in unilateral vocal fold paralysis—a case study. *Journal of Voice.* 2009;23:639-645.

**200.** Pedersen JR, Johannsen P, Bak CK, Kofoed B, Saermark K, Gjedde A. Origin of human motor readiness field linked to left middle frontal gyrus by MEG and PET. *Neuroimage.* 1998;8:214-220.

**201.** Hirano S, Kojima H, Naito Y, et al. Cortical dysfunction of the supplementary motor area in a spasmodic dysphonia patient. *American journal of otolaryngology.* 2001;22:219-222.

**202.** Peck KK, Galgano JF, Branski RC, et al. Event-related functional MRI investigation of vocal pitch variation. *Neuroimage.* 2009;44:175-181.

**203.** Belin P, Zatorre RJ, Lafaille P, Ahad P, Pike B. Voice-selective areas in human auditory cortex. *Nature.* 2000;403:309-312.

**204.** Zarate JM, Wood S, Zatorre RJ. Neural networks involved in voluntary and involuntary vocal pitch regulation in experienced singers. *Neuropsychologia.* 2010;48:607-618.

**205.** Bamiou D-E, Musiek FE, Luxon LM. The insula (Island of Reil) and its role in auditory processing: literature review. *Brain research reviews.* 2003;42:143-154.

**206.** Riecker A, Ackermann H, Wildgruber D, Dogil G, Grodd W. Opposite hemispheric lateralization effects during speaking and singing at motor cortex, insula and cerebellum. *Neuroreport.* 2000;11:1997-2000.

**207.** Ito M. Mechanisms of motor learning in the cerebellum. *Brain research.* 2000;886:237-245.

**208.** Schiller F. *Paul Broca: Founder of French anthropology, explorer of the brain*: Oxford University Press, USA; 1992.

**209.** Simonyan K, Saad ZS, Loucks TM, Poletto CJ, Ludlow CL. Functional neuroanatomy of human voluntary cough and sniff production. *Neuroimage.* 2007;37:401-409.

**210.** Mckay LC, Evans KC, Frackowiak RS, Corfield DR. Neural correlates of voluntary breathing in humans. *Journal of Applied Physiology.* 2003;95:1170-1178.

**211.** Perry DW, Zatorre RJ, Petrides M, Alivisatos B, Meyer E, Evans AC. Localization of cerebral activity during simple singing. *Neuroreport.* 1999;10:3979-3984.

**212.** Brown S, Martinez MJ, Hodges DA, Fox PT, Parsons LM. The song system of the human brain. *Cognitive Brain Research.* 2004;20:363-375.

**213.** Kleber B, Birbaumer N, Veit R, Trevorrow T, Lotze M. Overt and imagined singing of an Italian aria. *Neuroimage.* 2007;36:889-900.

**214.** Simonyan K, Ludlow CL. Abnormal activation of the primary somatosensory cortex in spasmodic dysphonia: an fMRI study. *Cerebral Cortex.* 2010:bhp023.

**215.** Simonyan K, Tovar-Moll F, Ostuni J, et al. Focal white matter changes in spasmodic dysphonia: a combined diffusion tensor imaging and neuropathological study. *Brain.* 2008;131:447-459.

**216.** Ali SO, Thomassen M, Schulz GM, et al. Alterations in CNS activity induced by botulinum toxin treatment in spasmodic dysphonia: an H215O PET study. *Journal of Speech, Language, and Hearing Research.* 2006;49:1127-1146.

**217.** Kiyuna A, Maeda H, Higa A, Shingaki K, Uehara T, Suzuki M. Brain activity related to phonation in young patients with adductor spasmodic dysphonia. *Auris Nasus Larynx.* 2014;41:278-284.

**218.** Rektorova I, Mikl M, Barrett J, Marecek R, Rektor I, Paus T. Functional neuroanatomy of vocalization in patients with Parkinson's disease. *Journal of the neurological sciences.* 2012;313:7-12.

**219.** Liotti M, Ramig L, Vogel D, et al. Hypophonia in Parkinson's disease neural correlates of voice treatment revealed by PET. *Neurology.* 2003;60:432-440.

**220.** Narayana S, Fox PT, Zhang W, et al. Neural correlates of efficacy of voice therapy in Parkinson's disease identified by performance–correlation analysis. *Human brain mapping.* 2010;31:222-236.

**221.** Joshi A, Jiang Y, Stemple JC, Archer SM, Andreatta RD. Induced unilateral vocal fold paralysis and recovery rapidly modulate brain areas related to phonatory behavior: a case study. *Journal of Voice.* 2011;25:e53-e59.

# CHAPTER 2

# IMPACT OF VAGAL NERVE STIMULATION ON OBJECTIVE VOCAL QUALITY

This chapter will present the readers with the results of evaluation of impact of VNS on the vocal quality[1].

**Abstract**

**Objective.** The purpose of this study was to determine the impact of VNS on the vocal quality using the DSI. It was hypothesized that the objective vocal quality and other vocal characteristics are disordered in comparison with an age- and gender-matched control group. In addition, the acoustic vocal parameters were compared during three conditions: at rest, during normal stimulation, and raised stimulation. A significant relation between the amount of stimulation and the presence of disturbed acoustic parameters was hypothesized.

**Methods.** Subjective (auditory-perceptual evaluation and voice handicap index) and objective (aerodynamic, vocal range, acoustic measurements and determination of the DSI) measurements were used to determine the vocal quality in 13 subjects with VNS in three different conditions (at rest and during normal and raised stimulation) and the age- and gender-matched control group.

**Results.** The subjects with VNS had a disordered perceptual vocal quality mainly characterized by the presence of a moderate roughness and slight breathiness, and the objective vocal quality by means of the DSI value is -2.4. During stimulation and especially during raised stimulation, the fundamental frequency is significantly increased. However, the subjects experienced no psychosocial handicapping effect of the vocal quality on the quality of life.

**Conclusions.** Subjects with VNS have typical vocal characteristics. Ear, nose, and throat specialists and voice therapist must be aware of the presence of this vocal pattern at rest and during normal and raised stimulation. Especially, professional voice users and elite vocal performers must be informed before implantation.

# 1. Introduction

VNS is used in patients with refractory epilepsy unsuitable for conventional resective surgery or not adequately controlled by drugs [193]. In this procedure, an electrode is implanted in the neck around the left VN and activated by a pulse generator implanted subcutaneously in the left infraclavicular region. Both frequency and amplitude of the stimulus can be programmed individually to decrease the frequency and severity of epileptic seizures depending on the desired effect on the decrease in severity of the seizures [56]. Long-term studies of VNS efficacy showed a seizure frequency reduction rate usually >50% both in adults and in the population [193]. Side effects related to the delivery of the stimulation pulse are mild but very common. Coughing, dyspnea, tonsillar pain, paresthesias, dysphagia, and voice problems (mostly hoarseness) have been reported by several authors. On the basis of the data from an extensive literature review (Table 2.1), it is obvious that both the laryngeal physiological characteristics, the aerodynamic MPT and acoustic parameters, and the (self-perceived) perceptual vocal characteristics are disordered.

With respect to the interpretation of the impact of the VNS, different results regarding the aerodynamic parameter MPT are noticed. Although Shaw et al [194] reported a reduced MPT after implantation, Shaffer et al [29] measured no significantly reduced MPT in subjects with VNS. Several assessment techniques were used to characterize the laryngeal and vocal changes as presented in Table 2.1.

Several authors used videolaryngostroboscopy and found varying degrees of left vocal fold abduction and adduction during stimulation [15, 56, 194-196], contraction of the left hemilarynx [56], tension of the left ventricular vocal fold [15], spasmodic contraction of the intrinsic laryngeal muscles [197, 198], and paresis or reduction of the mobility of the left vocal fold in the postsurgical condition [194, 199].

Abnormal electromyographic results (left thyroarytenoid and cricothyroid muscles) and decreased perceptual vocal characteristics were found in the study of Shaffer et al[29]. Moreover, an increased impact of the vocal changes after VNS on the QOL was reported by several researchers [15, 194, 200, 201]. A decrease of the MPT after implantation8 and during stimulation [29] and increased acoustic parameters (increased jitter

and shimmer in the study of Charous et al [15] and increase in F0 during stimulation in the study of Kersing et al [197] and Lundy et al [56] were also measured.

| Authors | N, Age, range (y) | Methods | Results |
|---|---|---|---|
| Lundy et al [56] | 5<br>Mean age: 36.2; range, 30-41<br>No controls | LV<br>Acoustic analysis<br>Airflow measurement | Varying degrees of VF abduction and adduction (stimulation at 20 and 40 Hz)<br>Higher levels of electrical stimulation (> 80 Hz) produced hemispasm of the larynx<br>Increased F0 and jitter with increasing frequency of stimulation<br>Increased glottal airflow from unstimulated condition to both 20 and 40 Hz and decreased at higher levels |
| Zumsteg et al [195] | 3<br>Mean age: 29.3; range, 26-34<br>No controls | LV | Left VF adduction during stimulation |
| Charous et al[15] | 5<br>No controls | Questionnaire<br>LV<br>Acoustic analysis<br>/i/ | Permanent or consistent change of the voice (with lower pitch, rougher, more strained, breathy and softer)<br>Paramedian position of left VF, VF tensing and loss of mucosal wave<br>Jitter and Shimmer increased at rest and during VN stimulation |
| Kersing et al [197] | 7<br>Range, 21-45<br>No controls | LV | Adductory spasm of either the ipsilateral VF or the vestibular VF |
| Santos [196] | 18<br>Mean age: 28; range, 4-73<br>No controls | LV | No permanent paresis/paralysis or hoarseness |
| Zalvan et al [199] | 4<br>Mean age: 15; range, 3-28<br>No controls | LV | Temporary VF paresis 1-wk after surgery (n = 4; related to the surgical manipulation) |
| Shaw et al [194] | 13<br>Range, 25-84<br>No controls | LV<br>MPT | VF mobility abnormalities at 2 wk (n=6)<br>3-mo postsurgery VF paresis (n=5)<br>Reduced in all subjects after implantation |
| Ardesch et al [198] | 8<br>Mean age, 3; range, 15-60<br>No controls | LV | Adductory spasm of ipsilateral VF or the vestibular fold |
| Felisati et al [200] | 14<br>mean age: 41<br>No controls | LV | Three different laryngeal patterns: (1) left VF palsy at baseline and during stimulation (n=4), (2) left VF palsy at baseline and left VF adduction during stimulation (n=7), (3) Symmetric pattern at the baseline and constant left VF adduction during stimulation (n=3) |
| Shaffer et al [29] | 10<br>Control group | VHI<br>Consensus perceptual evaluation<br>During connected speech<br>LV<br>LEMG<br>MPT | Increased self-perceived vocal handicap in patients with VN stimulation<br>Higher severity scores of voice problems during VM stimulation as compared with rest/ more roughness at rest and during stimulation/more breathiness during stimulation/more strain/pitch disturbances<br>Reduced VF mobility, completely or partially during stimulation (n=9), evidence of false VF hyperfunctional overadduction and supraglottic hyperadduction (as seen in muscle tension dysphonia)<br>Abnormal LEMG results (thyroaryntenoid and cricothyroid muscles)<br>Not significantly reduced in subjects with VNS |

**Table 2.1: Literature review regarding the impact of VNS on vocal quality.** Abbreviations: LV: laryngeal videostroboscopy, VF:vocal fold, VN: vagal nerve, MPT: Maximum Phonation Time; LEMG: laryngeal electromyography.

The few studies that focused on voice in patients treated with VNS reported limited data regarding the perceptual and objective vocal quality. Only in one study [15], an acoustic analysis (with a determination of the jitter and shimmer) and a consensus perceptual evaluation were performed. Moreover, most studies mentioned small patient series with varying ages and without an age- and gender-matched control group. To the best of our knowledge, no study has previously investigated the vocal range (frequency in Hertz) and amplitude (in decibel) and the objective vocal quality using a multiparameter approach by means of the dysphonia severity index (DSI) in subjects with a VNS. The more negative the patient's index, the worse is his or her vocal quality [202]. The use of a multivariate approach as a complex phenomenon is not new in health care. The body mass index is a good example of the power of combining variables. Moreover, multivariate techniques prove to be useful in voice research too as shown by the voice range profile index for children [203].

The main purpose of the present controlled study was to determine the objective vocal quality (by means of the DSI) at rest in 13 subjects treated with VNS. Moreover, a perceptual consensus evaluation was performed, and the self-perceived impact of the vocal quality on the overall QOL was determined. On the basis of the literature data, a significant decrease in the objective vocal quality (decreased DSI value) together with disordered perceptual vocal characteristics and an impact of the voice disorder on the QOL was hypothesized. In addition, the acoustic vocal parameters were compared during three conditions of stimulation. A significant relation between the amount of stimulation and the presence of disturbed acoustic parameters was hypothesized.

## 2. Methods and materials

This study was approved by the Human Subjects Committee of the University of Ghent (B670201318342, project 2013/694). Written informed consent was obtained from each participant.

### Subjects

Thirteen patients, seven men and six women with a mean age of 42.8 years (range, 24–57 years), all enrolled at the epilepsy clinic of the Ghent University Hospital, agreed to participate in this study. On

the basis of an extensive presurgical evaluation by a multidisciplinary epilepsy team, all patients were considered unsuitable candidates for resective surgery because of non- localizing findings or localization of the epileptic focus in functional brain tissue. The presurgical evaluation protocol has been previously described in the studies by Boon et al [204] and Vonck et al [205]. All patients were treated with chronic anti epileptic drug polytherapy. The surgical implantation procedure of the neurocybernetic prosthesis system [206, 207]and the ramping-up procedure of the stimulator have been described previously. The individual patient characteristics and stimulus characteristics are summarized in Table 2.2.

| Patient | Gender | Age (y) | Years of implantation | Stimulus characteristics | | | |
|---|---|---|---|---|---|---|---|
| | | | | ON/OFF | Frequency (Hz) | Intensity (mA) | Duration (μs) |
| 1 | F | 47 | 11 | 7s / 20s | 20 | 2,75 | 500 |
| 2 | F | 57 | 11 | 30s / 5min | 20 | 3,00 | 500 |
| 3 | F | 24 | 0 | 30s / 10min | 30 | 1,50 | 500 |
| 4 | M | 38 | 3 | 14s / 5min | 20 | 2,50 | 250 |
| 5 | M | 46 | 9 | 30s / 5min | 30 | 2,50 | 500 |
| 6 | M | 40 | 0 | 30s / 10min | 30 | 1,00 | 500 |
| 7 | F | 42 | 1 | 30s / 10min | 20 | 0,25 | 250 |
| 8 | F | 32 | 3 | 30s / 10min | 30 | 2,25 | 500 |
| 9 | M | 50 | 14 | 30s / 5min | 20 | 2,25 | 500 |
| 10 | M | 25 | 0 | 30s / 10min | 30 | 1,50 | 500 |
| 11 | M | 52 | 0 | 30s / 10min | 30 | 1,25 | 500 |
| 12 | F | 52 | 2 | 30s / 5min | 30 | 2,50 | 500 |
| 13 | M | 49 | 9 | 30s / 10min | 15 | 2,25 | 250 |

**Table 2.2: Characteristics of the patients (Gender, Age and Years of implantation) and Stimulus (Frequency, Intensity, and Duration of the Stimulus).**

The gender- and age-matched control group without VNS consisted of 13 adult subjects (seven men and six women) with a mean age of 42.8 years (range, 24–57 years). The independent Student t test showed no significant age and gender differences between the subjects in the experimental group and those

in the control group. All subjects had no history of neurologic disorders and voice disorders and were parents consulting the University Voice Clinic for a vocal complaint of their child.

## Methods

The same subjective (questionnaire and consensus perceptual evaluation) and objective voice assessment (aerodynamic and acoustic analyses, vocal performance, and determination of the DSI) were determined in both the experimental and the control groups. The acoustic parameters were used to measure the impact of the three conditions of stimulation (at rest and during normal and increased stimulation).

### *Questionnaire*

Subjective self-evaluation of the voice was performed using the voice handicap index (VHI)[208]. This standardized questionnaire was used to measure the subject's perceptions of the psychosocial impact of the vocal problem during daily life. The VHI score (with physical, functional, and emotional subscales) varies between 0 and 120 (maximum perceived disability due to vocal difficulties). A score of <20 on the different subscales indicates no impact of the self-perceived vocal quality on the QOL. A score between 20 and 40 and between 40 and 60 indicates the presence of a self-perceived vocal disability or significant vocal disability. A score of >60 indicates the presence of a vocal handicap.

### *Consensus perceptual evaluation*

A perceptual rating of the voice during connected speech was judged by two speech-language pathologists (L.B. and N.P.) using the GRBASI scale. The GRBAS scale consists of five well-defined parameters: G (overall grade of hoarseness), R (roughness), B (breathiness), A (asthenic), and S (strained)[209]. A sixth parameter I for instability of the voice was added to the original scale [210]. A four-point grading scale (0 = normal, 1 = slight, 2 = moderate, and 3 = severe) was used to indicate the grade of every parameter (concordance values were 84%).

### *Aerodynamic voice evaluation*

The MPT (seconds) was measured (at rest condition) on the basis of two test trials with the vowel /a/, sustained at the subject's habitual loudness and pitch in sitting position.

### *Vocal range*

Frequency and intensity range were measured (at rest condition) with the voice range profile for the Computerized Speech lab (CSL) manufactured by Kay Elemetrics [211]. The procedure by Helen et al14 was used. The subjects were instructed to inhale in a comfortable way and produce the vowel /a/ for at least 2 seconds, using a habitual pitch and loudness, a minimal pitch (F-low), a minimal intensity (I-low), a maximal pitch (F-high), and a maximal intensity (I-high).

### *Acoustic analysis*

For the determination of the acoustic parameters, the Multidimensional Voice Program for the CSL (Kay Telemetric) was used. The subjects were asked to sustain the vocal /a/ in a comfortable way. A midvowel segment from 3 seconds registered with a sample rate of 50 000 Hz was used for analysis. The parameters, jitter (%), shimmer (dB), F0 (Hz), harmonics-to-noise ratio (HNR), and amplitude/frequency tremor response instability (%), were determined at rest and during normal and increased stimulation output condition of the VNS.

### *Dysphonia severity index*

The DSI is based on the weighted combination of the following set of voice measurements: MPT (seconds), highest frequency (F-high in Hertz), lowest intensity (I-low in decibel), and jitter (%). The DSI is constructed as 0.13xMPT + 0.0053xF0-high - 0.26xI-low - 1.18xjitter + 12.4. The vocal parameters were determined during the rest position of the VNS. The DSI score ranges from +5 to 5, respectively, corresponding with normal and severely dysphonic voices. The more negative the DSI, the worse is the patient's vocal quality [202].

### *Statistical analysis*

IBM SPSS 22 statistical package (IBM Corp, Armonk, NY) was used for statistical analysis of the data. To determine the significance level of difference of the continuous variables between the experimental and control groups, the Mann-Whitney U test was applied. The Fisher exact test was performed to determine the significance level of the GRBASI scale. The Wilcoxon matched-pair signed rank test was used to determine the difference between the three conditions of stimulation (rest, normal stimulation, and raised stimulation). A probability level of <0.05 was considered to be significant.

## 3. Results

## Vocal quality at rest in subjects with VNS

### *Questionnaire*

The results of the VHI are presented in Table 2.3. The total VHI score (P 1⁄4 0.005) and all the VHI sub- scales (physical, P 1⁄4 0.004; functional, P < 0.001; and emotional, P < 0.001) were significantly different between the subjects with a VNS and the control group. In the VNS group, 54% (7 of 13 subjects) experienced vocal discomfort of the self-perceived vocal quality on the QOL and 46% (6 of 13 subjects) experienced no vocal discomfort of the voice on the QOL. In the control group, none of the subjects (n = 13) experienced vocal discomfort of the self-perceived vocal quality on the QOL.

| VHI subscales | VNS group | | | Control group | | | Level of significance (P) |
|---|---|---|---|---|---|---|---|
| | Mean±SE | SD | Range | Mean±SE | SD | Range | |
| VHI physical | 10±2 | 6 | 2-20 | 4±1 | 4 | 0-13 | 0.004* |
| VHI functional | 8±1 | 5 | 1-19 | 2±0 | 1 | 0-4 | <0.001* |
| VHI emotional | 5±1 | 5 | 0-18 | 0±0 | 0 | 0-0 | <0.001* |

**Table 2.3:Results of the Voice Handicap Index subscales in the VNS group and the control group.** The level of significance (p value) for the VHI subscales between the VNS and control group is provided. *p<0.05

### *Perceptual evaluation*

The median of the perceptual evaluation of the voices of the experimental and the control group using the GRBASI scale are presented in Table 2.4.

| Parameters | VNS group (n=13) Median (range) | Control group (n=13) Median (range) | Level of significance (p) |
|---|---|---|---|
| G | 2 (0-3) | 0 (0-0) | <0.001* |
| R | 2 (0-3) | 0 (0-0) | <0.001* |
| B | 1 (0-2) | 0 (0-0) | <0.001* |
| A | 0 (0-3) | 0 (0-0) | 0.096 |
| S | 0 (0-3) | 0 (0-0) | 0.015* |
| I | 0 (0-2) | 0 (0-0) | 1 |

**Table 2.4: The median of the perceptual evaluation of the voices of the experimental group with VNS and the control group.** The level of significance (p value) for each perceptual vocal parameter between the VNS and control group is provided. *p<0.05

Regarding the median scores, subjects with VNS showed the presence of a moderate grade of hoarseness (G2), roughness (R2), and the slight presence of breathiness (B1). Significantly, more hoarseness, roughness, breathiness, and strained vocal characteristics were judged in the subjects with VNS in comparison to the voices of the controls.

### *Aerodynamic and voice evaluation and vocal performance.*

The mean scores of the objective voice assessments of the experimental group with VNS and the control group are provided in Table 2.5. The MPT was significantly smaller in the subjects with VNS. The acoustic parameters, jitter, shimmer, and HNR, were significantly higher in the subjects with VNS, and the highest frequency or intensity was significantly smaller in comparison to the control group. Moreover, the overall objective vocal quality was significantly lower in the VNS group (DSI value of -2.4) in comparison to the control group (DSI value of +3.5).

| Parameters | Subjects with VNS | | | Control group | | | Level of significance p |
|---|---|---|---|---|---|---|---|
| | Mean±SE | SD | Range | Mean±SE | SD | Range | |
| MPT (s) | 13.5±0.8 | 2.9 | 10-20 | 22.5±1.2 | 4.2 | 16-20 | <0.001* |
| Jitter (%) | 1.8±0.3 | 1.2 | 0.54-3.75 | 0.77±0.2 | 0.5 | 0.25-1.96 | 0.016* |
| Shimmer (dB) | 0.6±0.1 | 0.4 | 0.25-1.85 | 0.25±0.02 | 0.08 | 0.12-0.40 | 0.003* |
| HNR | 0.15±0.01 | 0.05 | 0.11-0.28 | 0.12±0.0 | 0.02 | 0.09-0.15 | 0.044* |
| F0 (Hz) | 140±13 | 47 | 82-218 | 156±14 | 51 | 87-226 | 0.362 |
| F-high (Hz) | 365±41 | 147 | 155-622 | 879±90 | 325 | 208-1480 | <0.001* |
| F-low (Hz) | 104±10 | 36 | 65-196 | 105±8 | 30 | 65-147 | 0.880 |
| I-high (dB) | 96±2 | 6 | 80-103 | 105±1 | 4 | 95-111 | <0.001* |
| I-low (dB) | 63±1 | 3 | 59-68 | 60±1 | 4 | 54-65 | 0.034 |
| DSI | -2.4 ±0.45 | 1.63 | -4.87 to 0.93 | +3.52±0.5 | 1.96 | 1.32 to 6.95 | <0.001* |

**Table 2.5: Comparison of the results of the objective voice measures (MPT, acoustic parameters, vocal performance and DSI) between the subjects with VNS and the control group.** In addition the reference values are added. The level of significance (p value) for the parameters between the VNS and control group is provided. *p<0.05. Abbreviations: MPT: maximum phonation time, F-high/low: highest/lowest frequency, I-high/low: highest/lowest intensity, DSI: dysphonia severity index. * p<0.05, significant difference

| Parameters | Rest | | | Normal Stimulation | | | Raised Stimulation | | | Level of significance p |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±SE | SD | Range | Mean±SE | SD | Range | Mean±SE | SD | Range | |
| Jitter (%) | 1.8±0.3 | 1.2 | 0.54-3.75 | 2.34±0.56 | 2.01 | 0.61-6.67 | 2.42±0.33 | 1.21 | 0.63-4.36 | 0.058 |
| Shimmer (dB) | 0.6±0.1 | 0.4 | 0.25-1.85 | 0.6±0.09 | 0.31 | 0.16-1.09 | 0.72±0.08 | 0.28 | 0.37-1.27 | 0.368 |
| HNR | 0.15±0.01 | 0.05 | 0.11-0.28 | 0.19±0.02 | 0.07 | 0.12-0.32 | 0.20±0.01 | 0.04 | 0.14-0.28 | 0.794 |
| FTRI (%) | 1,44±1.07 | 3.87 | 0-14.28 | 0.42±0.11 | 0.40 | 0-1.08 | 1.06±0.38 | 1.36 | 0-3.8 | 0.754 |
| ATRI (%) | 2,23±0.79 | 2.84 | 0-8.59 | 4,53±1.62 | 5.84 | 0-17.8 | 4.56±1.44 | 5.19 | 0-15.21 | 0.521 |
| F0 (Hz) | 140±13 | 47 | 82-218 | 157±13 | 47 | 93-230 | 164±13 | 46 | 97-243 | 0.023* |
| Notes: The results of the Friedman test (level of significance) between the three conditions (at rest, normal stimulation, and raised stimulation) are provided. Abbreviations: SE, standard error of the mean; SD, standard deviation; FTRI, frequency tremor response instability; ATRI, amplitude tremor response instability.<br>*P < 0.05, significant difference. | | | | | | | | | | |

**Table 2.6: The results of the acoustic parameters ‘‘at rest’’ (no stimulation), during ‘‘normal stimulation,’’ and during ‘‘raised stimulation (output 0.25 mA higher)’’ in subjects with VNS (n=13).**

***Comparison of the acoustic parameters between the three conditions: at rest, during normal stimulation, and during increased output current stimulation of the VNS***

The results of the acoustic parameters ‘‘at rest’’ (no stimulation), during ‘‘normal stimulation,’’ and during ‘‘raised stimulation’’ in subjects with VNS (n = 13) are provided in Table 2.6. The results of the Friedman test (level of significance) between the three conditions (at rest, normal stimulation, and raised stimulation) showed a significant difference in the F0 during the three conditions. The F0 was highest during raised stimulation.

## 4. Discussion

The main purpose of the present controlled study was to determine the objective and subjective vocal characteristics in 13 subjects (mean age, 42.8 years) treated with VNS. As hypothesized, the results of this study showed a decreased objective vocal quality and the presence of perceptual voice disorders in the subjects with a VNS.

The VHI questionnaire demonstrated that there was a significant difference between the scores of the subjects with VNS on the self-perceived physical, functional, and emotional aspects of the vocal quality on the QOL compared to the control group. But, the interpretation of the results in the subjects with VNS revealed the absence of an impact of the self-perceived vocal characteristics on the QOL. The results of this study are totally in agreement with the VHI findings of the 10 subjects with VNS in the study of Shaffer et al[11] These researchers found a significant difference for both the physical and functional aspects of the vocal quality on the QOL between the subjects with and without VNS, but no impact of the vocal characteristics on the QOL was deduced. The lowest VHI scores in this study and in the study of Shaffer et al [29] were on the emotional sub- scale. The findings of this study parallel with the conclusion of Shaffer et al [29] that although some degree of physical and functional impact was generally perceived, most of the subjects did not perceive a significant emotional effect [29]. In the study of Charous et al [15] 95% of the patients noticed a change in the voice during VNS. Significantly, all subjects in the study of Charous et al [15]

indicated that they would elect to undergo implantation again even with the knowledge of the vocal effect. Because the subjects in this study perceived no emotional impact of the voice disability on the QOL, the same pattern, as in the study of Charous et al [15], is hypothesized.

The absence of the impact of the self-perceived vocal characteristics on the QOL by means of the VHI score was not reflected in both the perceptual judgment (mean G2 R2 B1 A0 S0 I0) and the objective DSI value of -2.4 (corresponding with a DSI % of 26). The auditory-perceptual impression of a moderate vocal disorder with the presence of a moderate rough- ness and slight breathiness was confirmed by the presence of a vocal quality value of -2.4 in the subjects with VNS in the rest condition (no stimulation). Hypothetically, one can assume that the vocal characteristics in the rest condition are influenced by the presence of the neurological disease of refractory epilepsy. A DSI of +1.6 (66%) is the cutting point separating normal from abnormal voices, whereas a DSI value of +2.5 corresponding with a DSI % of 75 reflects a normal vocal quality [212, 213]. A significant difference with the control group with a G0 R0 B0 A0 S0 I0, reflecting no perceptual vocal disorders, and DSI value of +3.5 (corresponding with a DSI % of 85) was measured. Comparison with data from other researchers is somewhat difficult because, to date, no other studies — using a multiparameter approach by means of the DSI value—were found. Analysis of the components of the DSI showed that the main variables responsible for the difference of the DSI value between the subjects with and without VNS were the MPT (seconds), jitter (%), and F-high (Hz). MPT is significantly shorter, jitter percentage is significantly greater (reflecting more hoarseness), and F-high is significantly lower, in subjects with VNS. Shaw et al [194] reported a reduced MPT previously in the study of 13 subjects with VNS, whereas Shaffer et al [29] measured no decrease in subjects with VNS. Hypothetically, one can assume that the differences in the number of participants (13 in this study and in the study of Shaw et al (Shaw, 2006 #306) vs 10 in the study of Shaffer et al [29]) and different speech sample (MPT during the sustained phonation of /a/ in this study vs not mentioned in the study of Shaffer et al (Shaffer, 2005 #309)) can make the MPT difference. Jitter percentage in the present study does parallel to those reported by Charous et al [15] in which an increased jitter at rest and during stimulation was measured.

Comparison of the acoustic parameters between the three conditions revealed a significant difference regarding the F0. The higher the stimulation, the higher the F0. Only the study of Lundy et al [56]

was found to confirm this finding. There are several muscle actions involved in raising vocal F0. The most important intrinsic laryngeal muscles are the cricothyroid muscle with elongation of the vocal ligament. But also, tension of the thyroarytenoid muscle and activity of the posterior cricoarytenoid muscle are needed [214]. The presence of complaints and vocal change during VNS may be caused by secondary stimulation of the superior laryngeal nerve [147, 150, 151, 215-217], indirect stimulation of the recurrent laryngeal nerve [150], or central nervous system side effect [218-220]. To what extent VNS has a direct impact on the intrinsic laryngeal muscles responsible for the increase of the vocal pitch is subject for further research.

The absence of laryngeal videostroboscopic recordings in relation to the perceptual characteristics and DSI value during the three conditions can be regarded as a limitation of this study. The use of laryngeal videostroboscopic evaluation was not possible in this vulnerable population. Also, a longitudinal study design (following the same subject before and several times after implantation) would have been a better choice but was not possible because of practical reasons. Moreover, comparison of the vocal characteristics of subjects having refractory epilepsy without VNS and with VNS would have provided valuable information and is subject for further research.

## 5. Conclusion

The results of this study demonstrated that subjects with VNS with a mean age of 42.8 years have a disordered perceptual vocal quality mainly characterized by the presence of a moderate roughness and slight breathiness. Moreover, the objective vocal quality by means of the DSI value is -2.4 (taking into account the normal DSI value of +1.6). During stimulation and especially during raised stimulation, the F0 is significantly increased. However, the subjects experienced no psychosocial handicapping effect of the vocal quality on the QOL.

## 6. References


**1.** Van Lierde K, Kryshtopava M, Gadeyne S, et al. Impact of Vagal Nerve Stimulation on Objective Vocal Quality, a Pilot Study. *Journal of Voice.* 2015;29:777. e779-777. e715.

**2.** Marras CE, Chiesa V, De Benedictis A, et al. Vagus nerve stimulation in refractory epilepsy: new indications and outcome assessment. *Epilepsy & Behavior.* 2013;28:374-378.

**3.** Lundy DS, Casiano RR, Landy HJ, Gallo J, Gallo B, Ramsey RE. Effects of vagal nerve stimulation on laryngeal function. *Journal of Voice.* 1993;7:359-364.

**4.** Shaw GY, Sechtem P, Searl J, Dowdy ES. Predictors of laryngeal complications in patients implanted with the Cyberonics vagal nerve stimulator. *Annals of Otology, Rhinology & Laryngology.* 2006;115:260-267.

**5.** Shaffer MJ, Szabo CA, Jackson CE, Simpson CB. Vagal nerve stimulation: clinical and electrophysiological effects on vocal fold function. *Annals of Otology, Rhinology & Laryngology.* 2005;114:7-14.

**6.** Zumsteg D, Jenny D, Wieser HG. Vocal cord adduction during vagus nerve stimulation for treatment of epilepsy. *Neurology.* 2000;54:1388-1389.

**7.** Charous SJ, Kempster G, Manders E, Ristanovic R. The effect of vagal nerve stimulation on voice. *The Laryngoscope.* 2001;111:2028-2031.

**8.** Santos PM. Evaluation of laryngeal function after implantation of the vagus nerve stimulation device. *Otolaryngology--Head and Neck Surgery.* 2003;129:269-273.

**9.** Kersing W, Dejonckere PH, van der Aa HE, Buschman HP. Laryngeal and vocal changes during vagus nerve stimulation in epileptic patients. *Journal of Voice.* 2002;16:251-257.

**10.** Ardesch J, Sikken J, Veltink P, Van Der Aa H, Hageman G, Buschman H. Vagus nerve stimulation for epilepsy activates the vocal folds maximally at therapeutic levels. *Epilepsy research.* 2010;89:227-231.

**11.** Zalvan C, Sulica L, Wolf S, Cohen J, Gonzalez□Yanes O, Blitzer A. Laryngopharyngeal dysfunction from the implant vagal nerve stimulator. *The Laryngoscope.* 2003;113:221-225.

**12.** Felisati G, Gardella E, Schiavo P, et al. Endoscopic laryngeal patterns in vagus nerve stimulation therapy for drug-resistant epilepsy. *European Archives of Oto-Rhino-Laryngology.* 2014;271:117-123.

**13.** Burgos J, Morris G, Inglese C, Smith P. Patient perception on voice changes with vagal nerve stimulation. *Epilepsia.* Vol 46: BLACKWELL PUBLISHING 9600 GARSINGTON RD, OXFORD OX4 2DQ, OXON, ENGLAND; 2005:227-227.

**14.** Wuyts FL, De Bodt MS, Molenberghs G, et al. The Dysphonia Severity IndexAn Objective Measure of Vocal Quality Based on a Multiparameter Approach. *Journal of Speech, Language, and Hearing Research.* 2000;43:796-809.

**15.** Heylen L, Wuyts FL, Mertens F, et al. Evaluation of the vocal performance of children using a voice range profile index. *Journal of Speech, Language, and Hearing Research.* 1998;41:232-238.

**16.** Boon P, Vonck K, De Reuck J, Caemaert J. Vagus nerve stimulation for refractory epilepsy. *Seizure.* 2001;10:448-455.

**17.** Vonck K, Van Laere K, Dedeurwaerdere S, Caemaert J, De Reuck J, Boon P. The mechanism of action of vagus nerve stimulation for refractory epilepsy: the current status. *Journal of clinical neurophysiology.* 2001;18:394-401.

**18.** Cyberonics I. *Implantation Procedure 2010.*Houston, TX, Webster.

**19.** Cyberonics I. *Physician's Manual 2012* Houston, TX, Webster.

**20.** Jacobson BH, Johnson A, Grywalski C, et al. The voice handicap index (VHI) development and validation. *American Journal of Speech-Language Pathology.* 1997;6:66-70.

**21.** Hirano M. *Clinical examination of voice*: Springer; 1981.

**22.** Timmermans B, De Bodt MS, Wuyts FL, Van de Heyning PH. Analysis and evaluation of a voice-training program in future professional voice users. *Journal of Voice.* 2005;19:202-210.

**23.** Elemetrics C. *Operational Manual, CSL Computerised Speech Lab Model 4300, Software Version 4.* 1992;Lincoln Park, NJ: Kay Elemetrics.

**24.** Van Lierde K, Claeys S, Dhaeseleer E, et al. The vocal quality in female student teachers during the 3 years of study. *Journal of Voice.* 2010;24:599-605.

**25.** Raes J, Wuyts FL, de Bodt M, Clement P. The Dysphonia Severity Index used with a percentage scale. *Stem-, spraak-en taalpathologie.* 2005;11.

**26.** Barlow SM, Andreatta RD. *Handbook of clinical speech physiology*: Singular Publishing Group; 1999.

**27.** Morrison M, Rammage L. The irritable larynx syndrome as a central sensitivity syndrome. *Canadian Journal of Speech-Language-Pathology and Audiology* 2010;34(4):282-289.

**28.** Phua S, McGarvey L, Ngu M, Ing A. Patients with gastro-oesophageal reflux disease and cough have impaired laryngopharyngeal mechanosensitivity. *Thorax.* 2005;60:488-491.

**29.** Ludlow CL. Central nervous system control of interactions between vocalization and respiration in mammals. *Head & neck.* 2011;33:S21-S25.

**30.** Hammer MJ. Design of a new somatosensory stimulus delivery device for measuring laryngeal mechanosensory detection thresholds in humans. *IEEE Transactions on Biomedical Engineering.* 2009;56:1154-1159.

**31.** Hammer MJ, Barlow SM. Laryngeal somatosensory deficits in Parkinson's disease: implications for speech respiratory and phonatory control. *Experimental brain research.* 2010;201:401-409.

**32.** Hammer MJ, Krueger MA. Voice-related modulation of mechanosensory detection thresholds in the human larynx. *Experimental brain research.* 2014;232:13-20.

**33.** Van Laere K, Vonck K, Boon P, Versijpt J, Dierckx R. Perfusion SPECT changes after acute and chronic vagus nerve stimulation in relation to prestimulus condition and long-term clinical efficacy. *Journal of Nuclear Medicine.* 2002;43:733-744.

**34.** Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. *Seizure.* 2008;17:699-706.

**35.** Cunningham JT, Mifflin SW, Gould GG, Frazer A. Induction of c-Fos and ΔFosB immunoreactivity in rat brain by vagal nerve stimulation. *Neuropsychopharmacology.* 2008;33:1884-1895.

# CHAPTER 3

# FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY OF BRAIN ACTIVITY ASSOCIATED WITH PITCH ADAPTATION DURING PHONATION IN HEALTHY WOMEN WITHOUT VOICE DISORDERS

This chapter will present the readers with the results for the fMRI study of brain activity associated with pitch adaptation during phonation in healthy women without voice disorders using the proposed fMRI protocol[1].

**Abstract**

**Objectives.** This functional magnetic resonance imaging (fMRI) study investigated the brain activity associated with pitch adaptation during phonation in healthy women without voice disorders.

**Study Design. T**his is an interventional prospective study.

**Methods.** Sixteen healthy women (mean age: 24.3 years) participated in a blocked design fMRI experiment involving two phonation (comfortable phonation and high-pitched phonation) and exhalation (prolonged exhalation) tasks. BrainVoyager QX Version 2.4 software was used for group-level general linear model analysis ($q[FDR] < 0.05$).

**Results.** Analyses showed a significant main effect of phonation with pitch adaptation compared with rest period in the bilateral precentral gyrus, superior frontal gyrus, posterior cingulate gyrus, superior and middle temporal gyrus, insula and cerebellum, left middle and inferior frontal gyrus, right lingual gyrus, cingulate gyrus, and thalamus. Statistical results also identified a significant main effect of exhalation compared with rest period in the bilateral precentral gyrus, cerebellum, right lingual gyrus, thalamus, and left supramarginal gyrus. In addition, a significant main effect of phonation was found in the bilateral superior temporal gyrus and right insula, as well as in the left midbrain periaqueductal gray for high-pitched phonation only.

**Conclusions.** We demonstrated that a blocked design fMRI is sensitive enough to define a widespread network of activation associated with phonation involving pitch variation. The results of this study will be implemented in our future research on phonation and its disorders.

# 1. Introduction

Human phonation is a laryngeal motor behavior that extends from reflexive laryngeal actions [22, 23] to highly skilled laryngeal sensorimotor control to support speech or singing [25]. A component of normal phonation is the variation of voice pitch (habitual, high, and low). Integration of the sensory input and laryngeal motor output is required for pitch adaptation during vocalization [66, 128]. Moreover, voice pitch variation necessitates coordination of the respiratory system, the articulatory system and subglottic pressure [176, 221-224]. With regard to the laryngeal system, pitch adaptation depends on the interaction between intrinsic and extrinsic laryngeal muscles[149, 225, 226]. Using a wide pitch range contributes to the richness of human voice expression. People with vocal problems (like functional dysphonia) often have limited pitch ranges, such as a high and narrow vocal pitch interval due to laryngeal postural problems during phonation[33-36]. The prevalence of functional dysphonia is 41% in the working-age population (25 – 64 years) and female professional voice users are predominantly affected (43% women vs. 36% men)[1]. This has been the rationale to investigate the neural control of voice pitch variation in women.

Neuroimaging techniques have become important tools to describe neural networks associated with laryngeal control of phonation [79, 167-169]. Recent fMRI[79, 80, 167, 168, 171] and PET[169] studies have shown that in order to understand the neural control of phonation, laryngeal control must be investigated distinct from the neural correlates for voluntary exhalation control and oral articulation. These studies have identified the sensorimotor cortex region (corresponding to BA 1, 2, 3, or 4), premotor cortex region (BA 6, 8), STG (BA 22,41, 42), insula (BA 13), cingulate gyrus/cortex, supramarginal gyrus (BA 40), lingual gyrus (BA 18, 19), thalamus, cerebellum, midbrain, and basal ganglia as key regions involved in non-disordered phonation[79, 80, 167-169, 171] (Figure 3.1). More specifically, as defined by functional brain imaging the sensorimotor cortex region functionally includes the primary motor cortex (BA 4) and primary somatosensory cortices (BA 1, 2, and 3), and is anatomically located on/in the pre/postcentral gyrus and central sulcus[172]. In addition, premotor cortex region functionally includes premotor cortex and SMA and anatomically located on/in the precentral gyrus and SFG, MFG, IFG[172]. The sensorimotor and premotor cortex regions, STG and insula have been identified as key areas involved in integration of sensory input and laryngeal motor output during vocalization [63, 136, 139, 178].

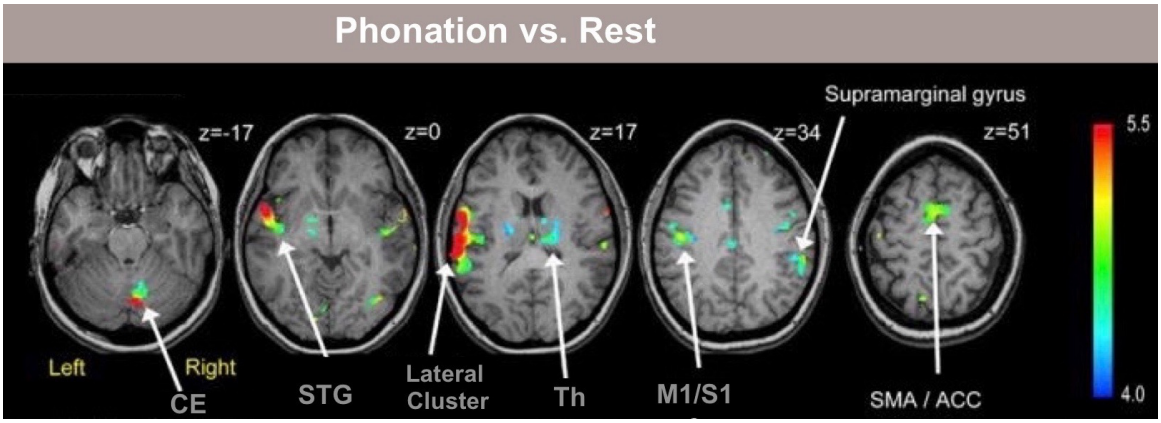


**Figure 3.1: FMRI activations for phonation (modified from Loucks et al, 2007)[21].** The Talairach template brain, z coordinates are given below each slice ($p<0.01$). Prominent activation is found in the left lateral cortex extending from the IFG, through the postcentral gyrus to the STG (BA 1–4, 6, 22, 44 ($z = 17$)), the right cerebellum ($z = -17$), the right supramarginal gyrus (BA 40; $z = 34$), the bilateral pre/postcentral gyrus (BA 3, 4, 6) in a region superior to the left ventrolateral cluster ($z = 34$), the SMA (BA 6, $z = 51$) and extended into the ACC. Prominent subcortical activation is found in ventral and medial nuclei of the right thalamus ($z=18$). (Abbreviations: BA: Brodmann area; IFG: inferior frontal gyrus, SMA: supplementary motor area; M1: primary motor cortex; S1: primary sensory cortex; ACC: anterior cingulate cortex).

Additionally, several studies have shown the neural basis of human pitch perception (*sensory* control) [227] in the STG and the neural basis of laryngeal *motor* control of vocal pitch modulation in the right IFG[176]. Studies such as these have advanced our understanding of the phonation control and vocal pitch modulation control. In addition, the fMRI study by Loucks et al [79] has demonstrated that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation, only a difference in STG activation was seen due to the auditory feedback. However, the *sensorimotor* integration control during vocal pitch changes remains poorly characterized. This is in part due to difficulties identifying the sensory, motor, and sensorimotor aspects of phonation control in an experiment. In addition, phonation demands simultaneous control of respiratory, laryngeal, and articulatory systems in production of various frequencies/pitches [176, 221-224]. Thus, investigations aiming to isolate the neural mechanisms of laryngeal *sensorimotor* control of pitch modulation are particularly challenging. In the fMRI study by Peck et al [176], production of neutral /uh/ sound at 3 vocal frequencies without labial and jaw movement was chosen. This

experimental paradigm evaluated laryngeal *motor* control of pitch adaptation in phonation with neutral vocal tract condition and minimal influence of jaw movements control and oral articulation. This approach was used in other fMRI studies with the production of /ə/ (schwa) sound with focus on laryngeal gestures only [80, 171] rather then sensory feedback. In other fMRI studies by Loucks et al [79], Haslinger et al [167], Simonyan et al [185], production of /i/ sound without labial and jaw movements was chosen. In this experimental paradigm laryngeal *sensorimotor* control of phonation was evaluated that requires precise sensory feedback and articulatory adjustment of the vocal tract during phonation. The approach that used production of /i/ sound to focus on *sensorimotor* control of phonation with minimal influence of oral articulation and jaw movements control was used in our study.

The aim of this study was two-fold: (1) to investigate the laryngeal neural control of phonation involving pitch (comfortable and high pitch) adaptation with minimal influence of voluntary respiratory control and oral articulation and (2) to examine usability of a blocked design fMRI method in defining the laryngeal neural control of phonation. In order to minimize the involvement of oral and pharyngeal muscles, we excluded tasks connected with laryngeal functions such as coughing, swallowing or speech. We implemented an experimental paradigm contrasting sustained phonation of unarticulated (i.e., without spreading the lips) sound /i/ with prolonged exhalation using subtraction approach during fMRI data analysis in order to focus on sensory feedback control of phonation. This approach is based on a study of Loucks et al [79] that showed that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation, only a difference in STG activation was seen due to the auditory feedback. These results were obtained by subtracting neural control of voluntary exhalation from neural control of phonation during fMRI data analysis with a subtraction approach[228, 229]. Additionally, the phonation tasks in this study explored the neural control associated with changes in pitch (comfortable and high). We hypothesized that a primary region related to phonatory activation would be the auditory cortex and that it can be observed as such by using fMRI. We focused on the STG because it has been identified as an integration area of sensory input and motor output during phonation[66, 68], specifically during error detection and correction involved in pitch processing[68, 136-139]. Furthermore, the STG is involved in auditory-vocal integration and processing of predicted and actual vocal output[140]. The findings may provide a foundation for future investigations of pitch adaptation in phonation and its disorders.

## 2. Materials and methods

### Participants

The study has been performed as an interventional prospective study. Sixteen healthy female, right-handed, native Flemish-speakers (21 – 45 years old, mean age: 24.3 years) with no history of neurological or psychiatric disease participated in the study. We reported the results of analyses performed on a cohort of 15 subjects. Subject 4 was excluded from analysis (data from functional scan was missing). Written informed consent was obtained from all participants. The same otorhinolaryngologist and speech therapist examined each subject clinically following a standard evaluation protocol. This protocol included the ENT evaluation, videostroboscopic examination[230], and the vocal quality evaluation by means of the DSI[202]. Each subject had normal laryngeal structure and function on videostroboscopy. All participants had a DSI value higher than +1.6 (mean DSI: +3.5) which constitutes a normal voice quality [202]. In addition, samples of voice based on the production of a sustained vowel /i/ were recorded during voice evaluation and the fundamental frequency (F0) and highest frequency (F-high) for each subject were assessed (mean F0: 211,6 and mean F-high: 799,3 of the vowel /i/). Before scanning, participants filled in a Pre-scan MRI-safety questionnaire, the Edinburgh Handedness Inventory measurement scale, and a Personal History Questionnaire. These questionnaires have been used to select participants who satisfy inclusion criteria, such as fMRI compatibility, participant characteristics, medical history, and lifestyle. After scanning, participants filled in a Post-Scan MRI-Checklist which asked for information on effects of the MRI equipment and its environment (i.e., magnetic field, acoustic noise) on scanned participants. The study confirmed to the code of ethics of the World Medical Association (Declaration of Helsinki)[231] and was approved by the Ethics Committee of Ghent University Hospital.

### Experiment

The fMRI experiment was performed in a block design, in which stimulation lasted 14.5 seconds followed by a period of rest ranging between 11 and 20 seconds (variable jittering). Jittered inter-stimulus (rest period) intervals were used to better determine the shape of whole hemodynamic responses function

(HRF) and to find a good baseline to evaluate response peaks[232]. The block of maximum 34.5 seconds was repeated 12 times for each condition. Each experimental condition had a total duration of 414 seconds. All participants were tested under three different conditions (Figure 3.2). These conditions were I. COMFORTABLE PHONATION: prolonged phonation of an unarticulated (i.e., without spreading the lips) vowel /i/ (similar to the "ee" in "see") on a habitual pitch level. II. HIGH-PITCHED PHONATION: prolonged phonation of the same unarticulated vowel /i/ using a high voice pitch. III. PROLONGED EXHALATION: voluntary sustained "unvoiced" oral exhalation. The order of conditions I to III were randomized in the different order for each participant.

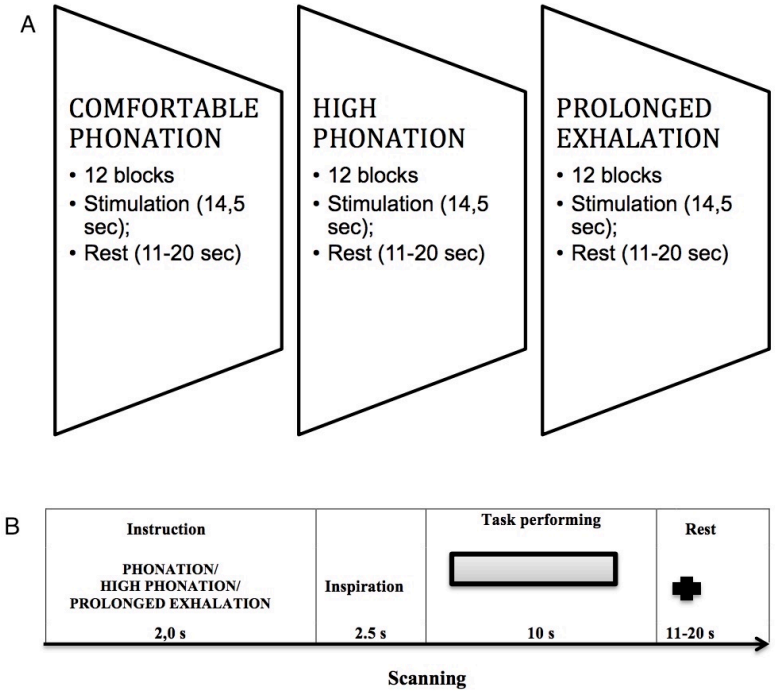


**Figure 3.2: Representation of the experimental conditions (A) and a single epoch (B).**

Periods during which the volunteers had to perform a task (either phonation or exhalation) were visually indicated during 10 seconds by a grey loading bar, whereas resting periods were indicated by a black cross. Before the actual task, two visual instructions were presented in the subject's native language indicating. First, the type of task was announced (i.e. in Dutch: "Comfortabele Stem", "Hoge Stem" or "Verlengde Uitademing") for 2 seconds. After this, a visual cue to start inspiration (i.e. in Dutch: "Inademen") which was presented during 2.5 seconds. These visual commands were generated using a

commercially available experiment generator (Presentation, Neurobehavioral Systems Inc., Albany CA, USA) and were projected onto a mirror on the head coil.

Before starting the fMRI scanning session, speech pathologist explained to all participants how to produce a sustained vowel /i/ during 10 seconds using a comfortable as well as a high pitch and to sustain exhalation for the same duration for the fMRI study. The project leader (SC) and MRI operator monitored tasks productions throughout the study through a control room speaker to assure that participants produce a phonation tasks correct.

### *Magnetic Resonance Imaging*

Magnetic resonance imaging was performed at 3 Tesla (Siemens Magnetom Trio, Erlangen, Germany) using the standard 32-channel head coil. Subjects were positioned head-first supine inside the magnet bore and fitted with a OptoACTIVE noise cancelling MRI headphone and a FOMRI-III noise cancelling microphone (OptoActive™, Optoacoustics Ltd, Moshav Mazor, Israel) in order to provide the highest level of noise reduction and self-monitoring of voice during phonation tasks. However, this OptoACTIVE system could not provide phonatory tasks recording without fMRI-related acoustic noise during image acquisition. The participant's head was immobilized in the standard head coil using neck cushions to minimize motion artifacts. The subjects were instructed to keep their jaw, lips and tongue motionless while performing the tasks and to keep their jaw slightly open in order to minimize movements during phonation (e.g. movements of orofacial muscles), which might cause artifacts during fMRI scanning. In addition, a vowel sound modification is produced by the first acoustic resonance of the vocal tract (R1) [233, 234], whose frequency depends on the vocal-tract articulatory shape and the boundary conditions at the glottis. In our study, participants reduced articulatory gestures due to sustained phonation of the vowel /i/ at a constant pitch during phonation tasks. Their performance was monitored throughout the experiment by the project leader (S.C.) and MRI operator (M.K.) to assure participants did not produce other sounds than the ones they were instructed to. Initially, an anatomical T1-weighted MR dataset covering the whole head at 1 $mm^3$ isotropic resolution was acquired (high-quality three-dimensional magnetization-prepared rapid acquisition with gradient echo (3D MPRAGE) images, repetition time: 1950

ms, inversion time: 1100 ms, echo time: 3.93 ms, flip angle: 12°). For functional imaging, a T2*-sensitive gradient-echo echo-planar imaging technique with an in-plane resolution of $2x2mm^2$ was used (repetition time: 2000 ms, echo time: 36 ms, flip angle: 70°, acquisition matrix: 96x128). Forty consecutive sections of 3-mm thickness with 0.5 mm gap between slices in an axial-to-coronal orientation were acquired. A total of 176 volumes were recorded for experimental run, resulting in a total investigation time of 25 minutes.

### *Image analysis steps*

Brain Voyager QX Version 2.4 software (Brain Innovation, Maastricht, The Netherlands) was used for fMRI data analysis[29]. Preprocessing included 3D motion correction, slice scan time correction, linear trend removal, and spatial smoothing on volume time course (VTC) files with a Gaussian kernel for the full width at the half maximum (FWHM) of 8 mm (the voxel size of resultant VTC was $3 \times 3 \times 3$ $mm^3$). Afterwards, functional datasets were coregistered to the anatomical dataset and transformed into Talairach space[235]. A statistical parametric map was calculated using the approach of the general linear model (GLM). For each experiment, a BrainVoyager protocol file (PRT) was derived, representing the onset and duration of the events for the different conditions and rest period as a baseline. From the created protocols, the design matrices for the calculation of the GLM were defined automatically. In order to account for hemodynamic response, each of the predictors was derived by convolution of the block design with a model for the two gamma hemodynamic response functions[229]. Previously, the GLM design matrix was improved by defining proper noise predictors using the Independent Component Analysis (ICA) approach[236]. After fitting the GLM [237], group t-maps were generated by invoking the RFX-ANCOVA (ANCOVA Random Effect Analysis) tool for the contrasts of COMFORTABLE PHONATION>REST; HIGH-PITCHED PHONATION>REST; PROLONGED EXHALATION>REST; COMFORTABLE PHONATION>PROLONGED EXHALATION; HIGH-PITCHED PHONATION>EXHALATION; HIGH-PITCHED PHONATION>COMFORTABLE PHONATION. Activation maps were generated by thresholding the statistical maps using the false discovery rate (FDR) approach ($q(FDR)<0.05$)[238].

## 3. Results

Figures 3.3, 3.4 and Table 3.1 summarize the main results of the GLM analysis. The GLM group analysis of phonation during pitch adaptation (COMFORTABLE PHONATION and HIGH-PITCHED PHONATION) compared to the rest period showed highly similar patterns of activity in the bilateral precentral gyrus, SFG, posterior cingulate gyrus, STG, middle temporal gyrus (MTG), insula and cerebellum, left MFG and IFG, right anterior cingulate/cingulate gyrus, lingual gyrus, and thalamus (Table 3.1 and Figure 3.3 A, B). Activities in the bilateral midbrain periaqueductal gray (PAG) were shown during the high pitch task only for the HIGH PHONATION>REST contrast rather than the COMFORTABLE PHONATION>REST contrast. However, the GLM analysis for the HIGH-PITCHED PHONATION>COMFORTABLE PHONATION contrast did not reveal any significant activation in the brain using FDR approach ($q(FDR)<0.05$). The GLM group analysis identified a significant main effect of exhalation for the PROLONGED EXHALATION>REST contrast in the bilateral precentral gyrus, cerebellum, right lingual gyrus and thalamus, and left supramarginal gyrus (Table 3.1 and Figure 3.3C). In addition, the GLM comparison for the COMFORTABLE PHONATION>PROLONGED EXHALATION and HIGH-PITCHED PHONATION>PROLONGED EXHALATION contrasts indicated a significant main effect in the bilateral STG and right insula (Table 3.1 and Figure 3.4). Activities in the bilateral STG and right insula were larger for the HIGH-PITCHED PHONATION>PROLONGED EXHALATION contrast, possibly reflecting a need for greater activity to produce higher frequency. Moreover, activity in the left midbrain PAG was present during the high pitch task only for the HIGH-PITCHED PHONATION>PROLONGED EXHALATION contrast rather than the COMFORTABLE PHONATION> PROLONGED EXHALATION contrast, possibly reflecting a greater activity for processing sensory input for higher vocal frequencies.

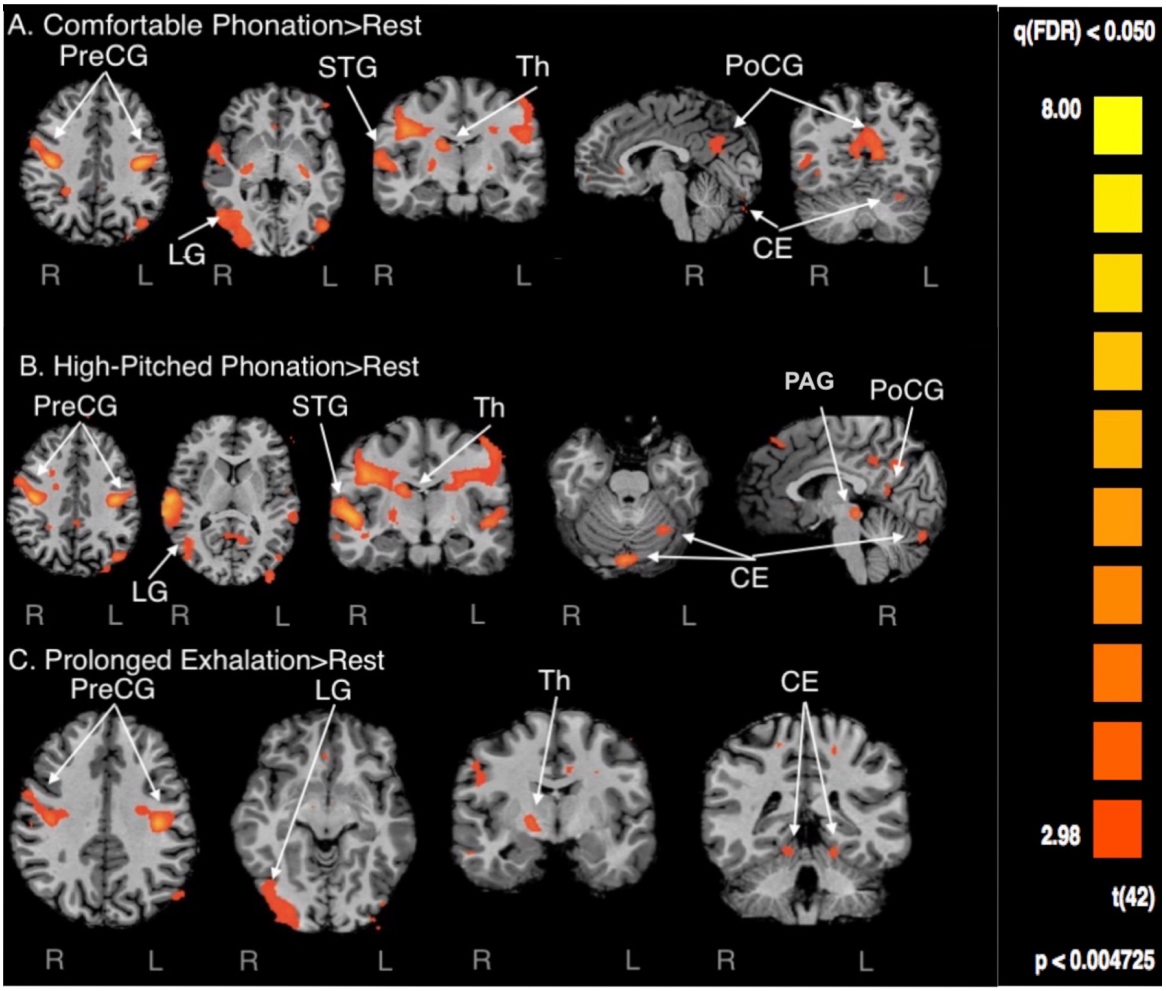


**Figure 3.3: Brain activation during phonation for the contrasts of COMFORTABLE PHONATION>REST (A), HIGH-PITCHED PHONATION>REST (B), and PROLONGED EXHALATION>REST (C).** The arrows indicate clusters of significant activation (q(FDR)<0,05). (Abbreviations: PreCG: precentral gyrus; STG: superior temporal gyrus; LG: lingual gyrus; PoCG: posterior cingulate gyrus; Th: thalamus; CE: cerebellum; PAG: periaqueductal gray).

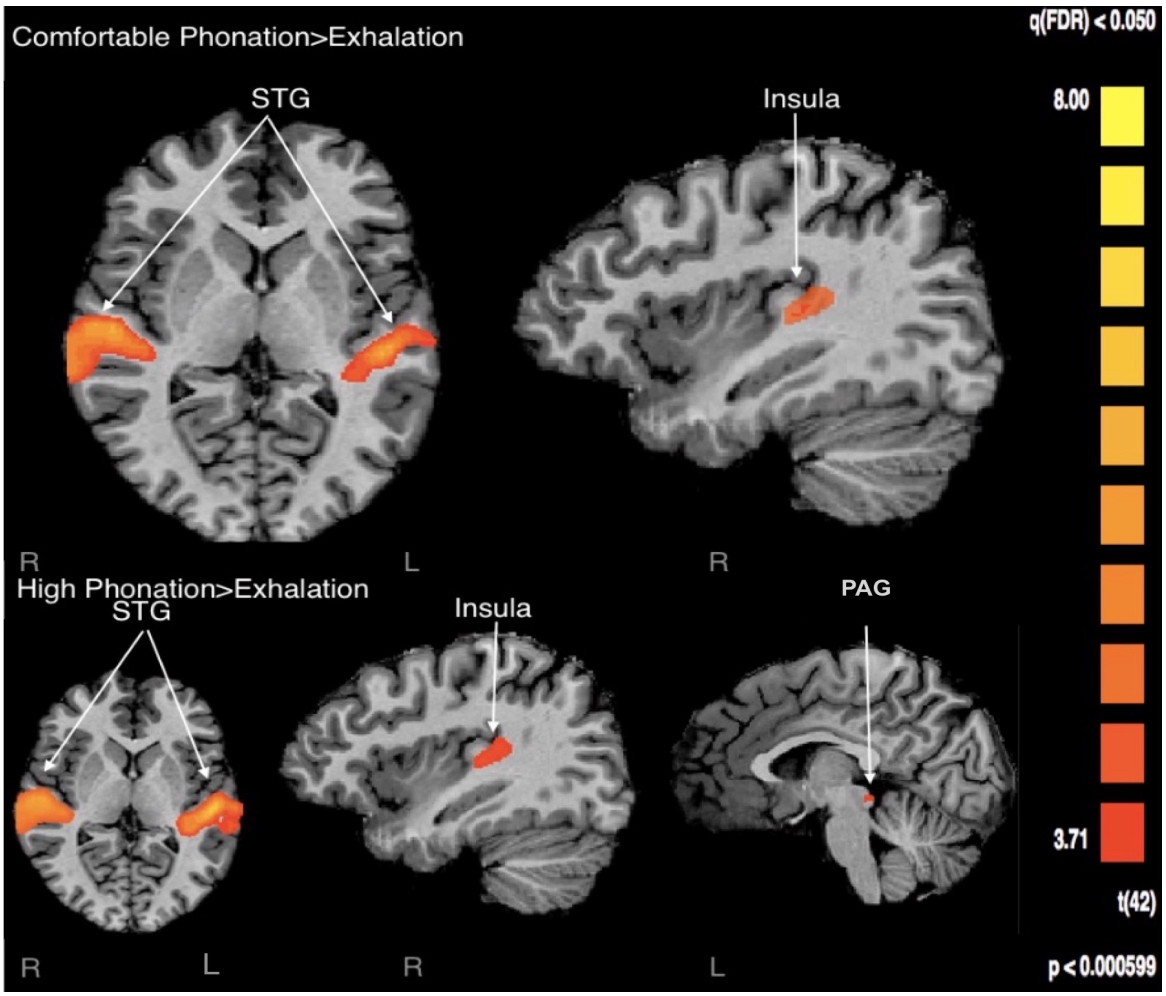


**Figure 3.4: Brain activation during phonation for the contrasts of COMFORTABLE PHONATION>PROLONGED EXHALATION and HIGH_PITCHED PHONATION>PROLONGED EXHALATION.** The arrows indicate clusters of significant activation (q(FDR)<0,05). (Abbreviations: STG: superior temporal gyrus; PAG: periaqueductal gray).

| Area | Brodmann, No | Cluster size ($mm^3$) | t(42)(peak) | Talairach coordinates x,y,z |
|---|---|---|---|---|
| **COMFORTABLE PHONATION>REST** | | | | |
| Right precentral gyrus | 4, 6 | 7304 | 8,1 | 41;-9;37 |
| Left precentral gyrus | 3-4,6 | 5298 | 8,0 | -43;-16;35 |
| Right superior frontal gyrus | 8 | 756 | 4,4 | 22;22;49 |
| Left superior frontal gyrus | 8 | 713 | 4,1 | -22;22;49 |
| Left middle frontal gyrus | 6 | 1875 | 4,3 | -28;17;51 |
| Left inferior frontal gyrus | 10,46 | 135 | 3,4 | -48;45;1 |
| Right cingulate gyrus | 31 | 596 | 4,7 | 25;-42;34 |
| Right posterior cingulate gyrus | 23 | 5931 | 4,1 | 6;-54;18 |
| Left posterior cingulate gyrus | 23 | | 4,7 | -3;-54;20 |
| Right lingual gyrus | 19 | 17152 | 5,3 | 37;-75;0 |
| Right superior temporal gyrus | 22, 41, 42 | 7154 | 6,6 | 57;-18;8 |
| Left superior temporal gyrus | 22, 42 | 722 | 4,1 | -53;-35;14 |
| Right middle temporal gyrus | 39 | 639 | 4,2 | 40;-68;15 |
| Left middle temporal gyrus (extends to the parietal lobe) | 39 | 9782 | 5,5 | -40;-75;18 |
| Right thalamus (ventral posterior lateral nucleus) | | 439 | 4,7 | 12;-11;18 |
| Right /Left Cerebellum | | 50/91 | 3,4/3,5 | 2;-81;-34/-28;-55;-25 |
| **HIGH-PITCHED PHONATION>REST** | | | | |
| Right precentral gyrus | 4,6 | 5443 | 7,3 | 45;-8;37 |
| Left precentral gyrus | 3-4,6 | 9838 | 7,6 | -45;-17;37 |
| Right superior frontal gyrus | 8 | 2836 | 5,2 | 8;33;49 |
| Left superior frontal gyrus | 8 | 361 | 3,6 | -8;33;49 |
| Left inferior frontal gyrus | 10 | 1247 | 5,3 | -52;45;-1 |
| Right anterior cingulate gyrus | 10 | 428 | 4,0 | 3;53;-2 |
| Right posterior cingulate gyrus | 23 | 4135 | 3,4 | 6;-54;18 |
| Left posterior cingulate gyrus | 31 | | 4,0 | -3;-52;23 |
| Right the lingual gyrus | 19 | 11085 | 4,6 | 39;-72;-10 |
| Left middle temporal gyrus (extends to the parietal lobe) | 39, 19 | 7305 | 5,6 | -46;-75;23 |
| Right superior temporal gyrus | 22, 41, 42 | 6310 | 6,7 | 53;-15;5 |
| Left superior temporal gyrus | 22, 42 | 916 | 4.0 | -56;-34;13 |
| Right thalamus (ventral posterior lateral nucleus) | | 771 | 3,9 | 12;-11;18 |
| Right/Left cerebellum | | 1080/302 | 5,5/3,8 | 2;-80;-24/-29;-56;-25 |
| Brainstem (left/right) | | 695 | 5,1 | -4/4;-27;-7 |
| **PROLONGED EXHALATION>REST** | | | | |
| Right precentral gyrus | 6 | 1932/2262 | 5,0/5,3 | 42;-10;32/46;3;11 |
| Left precentral gyrus | 4 | 2633 | 6,5 | -42;-17;37 |
| Right lingual gyrus | 19 | 7477 | 5,8 | 39;-70;-6 |
| Left supramarginal gyrus | 40 | 1176 | 4,7 | -42;-54;49 |
| Right thalamus | | 303 | 4,9 | 13;-7;6 |
| Right/Left cerebellum | | 55/247 | 3,6/4,8 | 11;-35;-14/-19;-34;-16 |
| **COMFORTABLE PHONATION>PROLONGED EXHALATION** | | | | |
| Right superior temporal gyrus | 22,41-42 | 4629 | 6,8 | 53;-22;8 |
| Left superior temporal gyrus | 22 | 2586 | 7,1 | -47;-24;6 |
| Right Insula | 13 | 330 | 5,7 | 31;-34;18 |
| **HIGH-PITCHED PHONATION>PROLONGED EXHALATION** | | | | |
| Right superior temporal gyrus | 22,41-42 | 10098 | 8,3 | 51;-21;7 |
| Left superior temporal gyrus and Insula | 21,22,41 | 8928 | 8,1 | -49;-23;4 |
| Right Insula | 13 | 652 | 5,7 | 33;-34;18 |
| Left Brainstem | | 81 | 4,3 | -5;-28;-7 |

**Table 3.1: Brain activation during phonation and exhalation.** Regions of significant activation are listed for each condition and for relevant contrasts between the conditions. Results are presented in Talairach space (q(FDR)<0,05).

# 4. Discussion

Healthy speakers use a wide pitch range during phonation whereas people with vocal problems often have limited pitch ranges, such as the predominantly high vocal pitch due to laryngeal postural problems during phonation in dysphonic patients[36-13]. This study investigated brain activity during phonation involving pitch adaptation in healthy women without voice disorders using fMRI. In our study, we reduced intra-group heterogeneity by including only adult healthy women. Moreover, we reasoned that we could use the results of this research in the future for the neural evaluation of functional dysphonia that tends to develop among adult women who use their voice as a professional tool[1]. We designed the study to investigate the neural laryngeal control of phonation during vocal pitch changes in isolation from articulatory and respiratory control. Integration of sensory input and laryngeal motor output is crucial for phonation, whereas during exhalation this sensory input is not needed[79]. We hypothesized that a primary region related to the activation associated with voice pitch adaptation would be the auditory cortex, more specifically the STG[66, 68, 136-140] and this would be observed by using fMRI. In our study, brain activity in related vocal pitch changes (COMFORTABLE PHONATION and HIGH-PITCHED PHONATION) was observed in the bilateral precentral gyrus, SFG, posterior cingulate gyrus, STG, MTG, insula and cerebellum, left MFG, IFG and inferior parietal lobe, right anterior cingulate/cingulate gyrus, lingual gyrus and thalamus. These results are corroborated by recent fMRI studies on phonation involving simple voice production tasks[68, 79, 80, 139, 167, 168]. The studies by Zarate and Zatorre [139] and Parkinson et al [68] have found activation during vocalization in the primary motor cortex, STG, anterior cingulate cortex, SMA, premotor cortex, insula, thalamus, putamen, and cerebellum. The study by Haslinger et al[167] has found activation in the primary sensorimotor cortex, premotor cortex, SMA, anterior cingulate cortex, inferior frontal cortex, anterior insula, STG, MTG, thalamus, cerebellum, midbrain, and basal ganglia. The study by Özdemir et al [168] has shown activation in the inferior primary sensorimotor cortex, IFG, and STG. A recent fMRI study by Loucks et al [79] has shown activation during phonation in the lateral sensory, motor and pre-motor regions in the left hemisphere, bilateral dorsolateral sensorimotor regions, right temporoparietal, cerebellar, and thalamic regions and the SMA and ACC.

The previously reviewed studies in addition to our study have observed activity in the brain areas during phonation that are specialized for different functions. More specifically, the MFG and IFG are responsible for the vocal motor planning [173-175]. Furthermore, the IFG is a key region involved in laryngeal *motor* control of vocal pitch modulation[176]. The MTG and STG are responsible for vocal self-monitoring [82] and voice processing[177], respectively. The STG is involved in *sensorimotor* integration for vocal production[239], an important component in vocal control. The insula is implicated in vocal monitoring as well as detection[179] and integration of sounds with a speaker's emotions and attitudes[180]. Cingulate cortex activity is associated with motor control [148] necessary for phonation, especially during pitch modulation. The cerebellum is involved in motor planning and coordination[181]. Bilateral activations in the precentral gyrus are related to larynx/phonation motor control area, described previously by Brown et al [80]. In addition, in the fMRI study by Loucks et al [79], activation during exhalation was indicated in the left ventrolateral cortex, precentral and postcentral gyri, right supramarginal gyrus, right lingual gyrus, right cerebellum and thalamus. In our study, a comparable pattern of responses was identified for exhalation, which included the bilateral precentral gyrus, cerebellum, left supramarginal gyrus, cingulate gyrus, right lingual gyrus, and right thalamus. In addition, the greater response in the bilateral STG and right insula differentiated phonation from exhalation in our experiment. These results are corroborated by Loucks et al [79] and Murphy et al [169] identified the greater response in the STG for auditory monitoring during vocalization.

In addition, to test whether sensory input affects brain activity during vocal pitch adaptation, participants performed phonation tasks with differing vocal pitch levels (COMFORTABLE PHONATION and HIGH-PITCHED PHONATION), where both auditory and somatosensory inputs were different. Activity in the midbrain PAG was present during the high pitch task only in the HIGH-PITCHED PHONATION>EXHALATION comparison, possibly reflecting a need for greater activity to filter less comforting sensory input for higher vocal frequencies before being sent to other areas of the brain. The PAG projects to the reticular formation of the lower brainstem, thus representing a neuroanatomical and functional relay station within the cingulate cortex-PAG-brainstem pathway. The PAG plays primarily a gating role in triggering a vocal response and modulating its intensity[240]. In addition, activities in the bilateral STG and right insula were larger for the HIGH-PITCHED PHONATION>PROLONGED

EXHALATION contrast, rather than for the COMFORTABLE PHONATION>PROLONGED EXHALATION contrast, possibly reflecting a need for greater activity in the integration of sensory information and vocal motor outputs that occurred with higher vocal effort required to control high-pitched phonation. However, differentiating comfortable pitch from high pitch for the HIGH-PITCHED PHONATION>COMFORTABLE PHONATION contrast did not show regions that were found in the cited work above [176] as this would cause a loss in statistical power in our study. The study by Peck et al [176] showed activation in the bilateral cerebellum, left IFG, left cingulate gyrus, and left posterior cingulate during high pitch in comparison to comfortable pitch while producing the "uh" sound at voxel probability threshold Pb0.005. However, our results may be explained based on the results of an fMRI experiment conducted by Parkinson et al [68]. They investigated the neural activations related to audio vocal responses using a pitch-shift perturbation paradigm (to pitch shifted vocalization). In this study, the STG activation was identified during pitch-shifted compared to non-shifted vocalization and did not reveal activation during non-shifted vocalization compared to pitch-shifted vocalization. It had been suggested, that a match between expected and actual output results in suppression in the auditory cortex and overlapping pattern of activations. On the other hand, a mismatch between expected and actual output results in an increase of sensitivity in the auditory cortex [142, 143]. We supposed that in our study a match between expected and actual output during phonation with different vocal pitch sound parameters would result in completely overlapping response patterns for phonation in the cerebral regions mentioned previously.

The findings in this study provide insight into phonation control. In our experiment, we were able to locate brain regions important to phonation control [63, 68, 136, 139, 141, 178, 241], and compared it to findings in the works cited above[63, 68, 136, 139, 141, 178, 241]. Moreover, we indicated regions (the bilateral precentral gyrus, SFG, posterior cingulate gyrus, STG, MTG, insula and cerebellum, left IFG, right cingulate, lingual gyrus, and thalamus) with robust activation during phonation. In our study we used a blocked designed paradigm that is effective for the detection of a widespread set of cortical and sub-cortical regions associated with phonation control in a healthy group. These data may be used as a template for future research on the neural evaluation of phonation and its disorders.

## 5. Conclusion

In our study, brain activity associated with pitch adaptation during phonation identified a network for motor, sensory and sensorimotor integration control of phonation. Our results have demonstrated that phonation involving pitch adaptation evoked activation in the sensorimotor and premotor cortex regions, bilateral cingulate gyrus, STG, MTG, insula and cerebellum, right lingual gyrus and thalamus. These findings are corroborated by recent fMRI studies on phonation involving simple voice production tasks. In addition, these results established that a blocked design fMRI is sensitive enough to define a neural network associated with phonation involving pitch variation. Importantly, our findings demonstrated that phonation (comfortable and high-pitched) evoked the largest activation in the bilateral STG and right insula providing a greater insight into the process of integration of multisensory input in laryngeal motor output during voice pitch variation. Moreover, greater activity in the STG and insula in high-pitched phonation possibly reflects a need for integration of sensory and motor output that occurred with higher vocal effort to control higher frequency phonation. Activation in the midbrain PAG for high pitch phonation may only be needed for processing the less comfortable sensory input resulting from higher vocal frequencies. During phonation in a comfortable pitch range compared to a high pitch range, no significant activations in the brain were revealed. This was possibly related to a match between expected and actual output during phonation, resulting in cancellation of sensory input, suppression in the auditory cortex, and overlapping pattern of responses for phonation in the cerebral regions. Understanding the process of integration of sensory input in laryngeal motor output provides a greater insight into normal phonation and its disorders. Future studies using blocked designed fMRI experiments in people with normal phonation and its disorders are recommended. It is interesting to extend our study with dysphonic patients.

## 6. References


**1.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2017;31:118. e121-118. e128.

**2.** Ludlow CL. Laryngeal Reflexes: Physiology, Technique, and Clinical Use. *Journal of Clinical Neurophysiology.* 2015;32:284-293.

**3.** Wyke B. Reflexogenic contributions to vocal fold control systems. In: Titze IR, Ronald CS, eds. *Vocal fold physiology: biomechanics, acoustics and phonatory control* The Denver Center for the Performing Arts, Inc., Denver, Colorado: College-Hill; 1983:138-141.

**4.** Ludlow CL. Central Nervous System Control of Voice and Swallowing. *Journal of Clinical Neurophysiology.* 2015;32:294-303.

**5.** Hickok G, Houde J, Rong F. Sensorimotor integration in speech processing: computational basis and neural organization. *Neuron.* 2011;69:407-422.

**6.** Larson CR, Sun J, Hain TC. Effects of simultaneous perturbations of voice pitch and loudness feedback on voice F0 and amplitude control. *The Journal of the Acoustical Society of America.* 2007;121:2862-2872.

**7.** Sundberg J. What's so special about singers? *Journal of voice.* 1990;4:107-119.

**8.** Bouhuys A, Mead J, Proctor DF, Stevens KN. Pressure□flow events during singing. *Annals of the New York Academy of Sciences.* 1968;155:165-176.

**9.** Davis PJ, Zhang SP, Winkworth A, Bandler R. Neural control of vocalization: Respiratory and emotional influences. *Journal of Voice.* 1996;10:23-38.

**10.** Peck KK, Galgano JF, Branski RC, et al. Event-related functional MRI investigation of vocal pitch variation. *Neuroimage.* 2009;44:175-181.

**11.** Finnegan EM, Luschei ES, Hoffman HT. Modulations in respiratory and laryngeal activity associated with changes in vocal intensity during speech. *Journal of Speech, Language, and Hearing Research.* 2000;43:934-950.

**12.** Poletto CJ, Verdun LP, Strominger R, Ludlow CL. Correspondence between laryngeal vocal fold movement and muscle activity during speech and nonspeech gestures. *Journal of Applied Physiology.* 2004;97:858-866.

**13.** Ludlow CL. Central nervous system control of the laryngeal muscles in humans. *Respiratory physiology & neurobiology.* 2005;147:205-222.

**14.** Honda K, Hirai H, Masaki S, Shimada Y. Role of vertical larynx movement and cervical lordosis in F0 control. *Language and Speech.* 1999;42:401-411.

**15.** Khoddami SM, Nakhostin Ansari N, Izadi F, Talebian Moghadam S. The assessment methods of laryngeal muscle activity in muscle tension dysphonia: a review. *The Scientific World Journal.* 2013;2013.

**16.** Angsuwarangsee T, Morrison M. Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice.* 2002;16:333-343.

**17.** Kooijman P, De Jong F, Oudes M, Huinck W, Van Acht H, Graamans K. Muscular tension and body posture in relation to voice handicap and voice quality in teachers with persistent voice complaints. *Folia phoniatrica et logopaedica.* 2005;57:134-147.

**18.** Lowell SY, Kelley RT, Colton RH, Smith PB, Portnoy JE. Position of the hyoid and larynx in people with muscle tension dysphonia. *The Laryngoscope.* 2012;122:370-377.

**19.** Van Houtte E, Van Lierde K, D'haeseleer E, Claeys S. The prevalence of laryngeal pathology in a treatment□seeking population with dysphonia. *The Laryngoscope.* 2010;120:306-312.

**20.** Haslinger B, Erhard P, Dresel C, Castrop F, Roettinger M, Ceballos-Baumann A. "Silent event-related" fMRI reveals reduced sensorimotor activation in laryngeal dystonia. *Neurology.* 2005;65:1562-1569.

**21.** Özdemir E, Norton A, Schlaug G. Shared and distinct neural correlates of singing and speaking. *Neuroimage.* 2006;33:628-635.

**22.** Loucks TM, Poletto CJ, Simonyan K, Reynolds CL, Ludlow CL. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. *Neuroimage.* 2007;36:131-143.

**23.** Murphy K, Corfield D, Guz A, et al. Cerebral areas associated with motor control of speech in humans. *Journal of Applied Physiology.* 1997;83:1438-1447.

**24.** Brown S, Laird AR, Pfordresher PQ, Thelen SM, Turkeltaub P, Liotti M. The somatotopy of speech: phonation and articulation in the human motor cortex. *Brain and cognition.* 2009;70:31-41.

**25.** Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cerebral Cortex.* 2008;18:837-845.

**26.** Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage.* 2006;31:1453-1474.

**27.** Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. *Neuroimage.* 2008;39:1429-1443.

**28.** Zarate JM, Zatorre RJ. Experience-dependent neural substrates involved in vocal pitch regulation during singing. *Neuroimage.* 2008;40:1871-1887.

**29.** Zarate JM, Wood S, Zatorre RJ. Neural networks involved in voluntary and involuntary vocal pitch regulation in experienced singers. *Neuropsychologia.* 2010;48:607-618.

**30.** Flagmeier SG, Ray KL, Parkinson AL, et al. The neural changes in connectivity of the voice network during voice pitch perturbation. *Brain and language.* 2014;132:7-13.

**31.** Lattner S, Meyer ME, Friederici AD. Voice perception: sex, pitch, and the right hemisphere. *Human brain mapping.* 2005;24:11-20.

**32.** Simonyan K, Ludlow CL. Abnormal activation of the primary somatosensory cortex in spasmodic dysphonia: an fMRI study. *Cerebral Cortex.* 2010:bhp023.

**33.** Miyapuram KP. Introduction to fMRI: experimental design and data analysis. 2008.

**34.** Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg M, Turner R. Event-related fMRI: characterizing differential responses. *Neuroimage.* 1998;7:30-40.

**35.** Parkinson AL, Flagmeier SG, Manes JL, Larson CR, Rogers B, Robin DA. Understanding the neural mechanisms involved in sensory control of voice production. *Neuroimage.* 2012;61:314-322.

**36.** Behroozmand R, Larson CR. Error-dependent modulation of speech-induced auditory suppression for pitch-shifted voice feedback. *BMC neuroscience.* 2011;12:54.

**37.** Guenther FH, Ghosh SS, Tourville JA. Neural modeling and imaging of the cortical interactions underlying syllable production. *Brain and language.* 2006;96:280-301.

**38.** Zarate JM, Zatorre RJ. Neural substrates governing audiovocal integration for vocal pitch regulation in singing. *Annals of the New York Academy of Sciences.* 2005;1060:404-408.

**39.** Remacle M. The contribution of videostroboscopy in daily ENT practice. *Acta oto-rhino-laryngologica Belgica.* 1995;50:265-281.

**40.** Wuyts FL, De Bodt MS, Molenberghs G, et al. The Dysphonia Severity IndexAn Objective Measure of Vocal Quality Based on a Multiparameter Approach. *Journal of Speech, Language, and Hearing Research.* 2000;43:796-809.

**41.** World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bulletin of the World Health Organization.* 2001;79:373.

**42.** Amaro E, Barker GJ. Study design in fMRI: basic principles. *Brain and cognition.* 2006;60:220-232.

**43.** Wolfe J, Garnier M, Smith J. Vocal tract resonances in speech, singing, and playing musical instruments. *HFSP journal.* 2009;3:6-23.

**44.** Sundberg J. Articulatory configuration and pitch in a classically trained soprano singer. *Journal of Voice.* 2009;23:546-551.

**45.** Talairach J, Tournoux P. *Co-planar Stereotaxic Atlas of the Human Brain: 3-dimensional Proportional System : an Approach to Cerebral Imaging.* 1st ed. Stuggart, Germany: Thieme; 1988.

**46.** Calhoun VD, Liu J, Adalı T. A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. *Neuroimage.* 2009;45:S163-S172.

**47.** Bullmore E, Brammer M, Williams SC, et al. Statistical methods of estimation and inference for functional MR image analysis. *Magnetic Resonance in Medicine.* 1996;35:261-277.

**48.** Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage.* 2002;15:870-878.

**49.** Galgano JF, Peck KK, Branski RC, et al. Correlation between functional MRI and voice improvement following type I thyroplasty in unilateral vocal fold paralysis—a case study. *Journal of Voice.* 2009;23:639-645.

**50.** Pedersen JR, Johannsen P, Bak CK, Kofoed B, Saermark K, Gjedde A. Origin of human motor readiness field linked to left middle frontal gyrus by MEG and PET. *Neuroimage.* 1998;8:214-220.

**51.** Hirano S, Kojima H, Naito Y, et al. Cortical dysfunction of the supplementary motor area in a spasmodic dysphonia patient. *American journal of otolaryngology.* 2001;22:219-222.

**52.** Schulz G, Varga M, Jeffires K, Ludlow C, Braun A. Functional neuroanatomy of human vocalization: an H215O PET study. *Cerebral cortex.* 2005;15:1835-1847.

**53.** Belin P, Zatorre RJ, Lafaille P, Ahad P, Pike B. Voice-selective areas in human auditory cortex. *Nature.* 2000;403:309-312.

**54.** Hickok G, Buchsbaum BR, Humphries C, Muftuler T. Auditory–motor interaction revealed by fMRI: speech, music, and working memory in area Spt. *Cognitive Neuroscience, Journal of.* 2003;15:673-682.

**55.** Bamiou D-E, Musiek FE, Luxon LM. The insula (Island of Reil) and its role in auditory processing: literature review. *Brain research reviews.* 2003;42:143-154.

**56.** Riecker A, Ackermann H, Wildgruber D, Dogil G, Grodd W. Opposite hemispheric lateralization effects during speaking and singing at motor cortex, insula and cerebellum. *Neuroreport.* 2000;11:1997-2000.

**57.** Picard N, Strick PL. Motor areas of the medial wall: a review of their location and functional activation. *Cerebral cortex.* 1996;6:342-353.

**58.** Ito M. Mechanisms of motor learning in the cerebellum. *Brain research.* 2000;886:237-245.

**59.** Dujardin E, Jürgens U. Afferents of vocalization-controlling periaqueductal regions in the squirrel monkey. *Brain research.* 2005;1034:114-131.

**60.** Heinks-Maldonado TH, Houde JF. Compensatory responses to brief perturbations of speech amplitude. *Acoustics Research Letters Online.* 2005;6:131-137.

**61.** Houde JF, Jordan MI. Sensorimotor Adaptation of Speech ICompensation and Adaptation. *Journal of Speech, Language, and Hearing Research.* 2002;45:295-310.

**62.** Toyomura A, Koyama S, Miyamaoto T, et al. Neural correlates of auditory feedback control in human. *Neuroscience.* 2007;146:499-503.

**63.** Parkinson AL, Korzyukov O, Larson CR, Litvak V, Robin DA. Modulation of effective connectivity during vocalization with perturbed auditory feedback. *Neuropsychologia.* 2013;51:1471-1480.

# CHAPTER 4

# BRAIN ACTIVITY DURING PHONATION IN WOMEN WITH MUSCLE TENSION DYSPHONIA: AN FMRI STUDY

This chapter will present the readers with the results for fMRI study of brain activity during phonation in women with MTD using the proposed fMRI protocol described in Chapter 3[1].

**Abstract**

**Purpose:** The main purposes of this fMRI study are 1) to investigate brain activity during phonation in women with MTD in comparison with healthy controls; and 2) to explain the neurophysiological mechanism of laryngeal hyperfunction/tension during phonation in patients with MTD.

**Methods:** Ten women with MTD and fifteen healthy women participated in this study. The fMRI experiment was carried out using a block design paradigm. Brain activation during phonation and exhalation was analyzed using Brain Voyager software.

**Results:** The statistical analysis of fMRI data has demonstrated that MTD patients control phonation by use of the auditory, motor, frontal, parietal, and subcortical areas similar to phonation control by healthy people. Comparison of phonation tasks in the two groups revealed *higher* brain activities in the precentral gyrus, inferior, middle and superior frontal gyrus, lingual gyrus, insula, cerebellum, midbrain, and brainstem as well as *lower* brain activities in the cingulate gyrus, superior and middle temporal gyrus, and inferior parietal lobe in the MTD group. No differences were found between two groups regarding exhalation control.

**Conclusions:** The findings in this study provide insight into phonation and exhalation control in patients with MTD. The imaging results demonstrated that in patients with MTD, altered (higher/lower) brain activities may result in laryngeal tension and vocal hyperfunction.

# 1. Introduction

The prevalence of functional dysphonia is 41% in the working- age population (25–64 years) seeking consultation in an ear, nose, and throat department. Female professional voice users are predominantly affected (43% women vs. 36% men) [1]. The term MTD is often used to describe functional voice disorder with increased vocal hyperfunction. Vocal hyperfunction can be defined as the involvement of excessive muscle force and physical effort during phonation [2]. It develops from incoordination of muscles or excessive muscle usage in phonation [3]. Causes of MTD include environmental (external) or systemic (internal) factors or stimuli. Common factors or stimuli are upper respiratory infection, second-hand smoke, LPR, significant vocal demands, or stressful life events [4]. In MTD, hyperfunctional vocal behavior is often a result of inappropriate compensatory strategies for muscle activities adopted in response to environmental or systemic stimuli [5]. However, the pathophysiological mechanism of MTD is not fully understood [5–9]. The major pathophysiological finding in patients with functional voice disorders is that the hyoid and larynx positions are higher in such patients than in controls [10]. The only muscles which may be affected in this context is the TH muscle which raises the larynx to the hyoid, the anterior belly of the digastric muscle, and the MH in the submental region which pull the hyoid upwards [40]. Van Houtte et al [18] have found TH muscle overactivity during phonation in patients with MTD compared to a healthy group. However, no studies have verified that the anterior belly of the digastric muscle and the MH muscle are consistently activated in MTD. Moreover, the neurophysiological background of functional voice disorder is currently unknown.

Human phonation can be defined as a laryngeal motor behavior that extends from reflexive and unlearned limbic laryngeal actions [22, 23] to highly skilled laryngeal sensorimotor control to support speech and/or singing [25]. Phonation requires coordination of the respiratory, laryngeal, and articulatory systems, and subglottic pressure [176, 221-224]. During development of phonation, and particularly of vocal quality, laryngeal motor control becomes increasingly skilled and rapid. Moreover, the balance of aerodynamic and muscle forces adapts to rapidly changing vocal requirements, including modulations of pitch, loudness, and rate. Based on preliminary data on voice and speech control, it is known that sensory feedback (auditory and somatosensory) [64] plays an important role in development of phonation (Figure 4.1 A)[115, 116]. However,

the sensory feedback control is too slow to support required rapid and skilled vocal movements. Most of these movements are pre-programmed. These programs require the generation of internal representations (neural 'model') of the sensorimotor transformations required to generate the set of motor commands that will execute a desired movement. Once this neural 'model' is learned, the internal system can then predict likely sensory consequences of a motor command prior to the arrival of actual sensory feedback. Thus, online feedback control is achieved primarily via the neural 'model' whereas actual feedback is used to train and update this neural 'model'. Hence, the neural 'model' plays an important role in executing rapid and skilled laryngeal vocal movements [24-26]. On the one hand, this neural 'model' reinforces or corrects the motor activation in the brain [26] to support rapid skilled vocal movements [24, 25]. On the other hand, the neural 'model' adjusts brain processing to the current sensory information to improve vocal performance [27]. Any changes in the larynx require adaptation and updating of these neural 'model' [26]. Feedback provides necessary information and plays a key role in learning, maintaining, and updating the neural 'model' and can also be used to correct overt prediction/feedback mismatch errors [28](Figure 4.1 A).

From a more fundamental neurobiological point of view, the modulation in sensory feedback brings about significant central neuroplastic changes [118, 119, 30]. Neural plasticity or brain plasticity is the ability of the CNS to change and adapt in response to environmental cues, experience, behavior, injury or disease. Neural plasticity can result from a change in function within a particular neural substrate in the CNS through alterations in neuronal excitability [31]. Changes in the function of a neural substrate can then alter behavior secondary to environmental influences such as experience, learning, development, aging, change in use, injury or response to injury such as unmasking due to the loss of surround inhibition with reduced afferent input [32-34]. Neural plasticity may alter the function of the original neural substrate used to produce a regular behavior [35]. Understanding how the brain adapts to a changing environment will provide insight into how this adaptation influences on the development of phonation and its disorders. A recent study has suggested an association between the internal representations/neural 'model' of the sensorimotor transformations and MTD [36]. However, there are no studies that evaluate neural correlates of phonation in MTD.

Neuroimaging techniques are objective tools recently used to describe neural pattern associated with control of normal vocalization [18, 37-47] and voice disorders [48-57]. Recent fMRI [37,38,40,41,49] and PET [39] studies have identified key regions involved in non-disordered phonation which located in the sensorimotor cortex region, premotor cortex region, STG, insula, cingulate gyrus/cortex, supramarginal gyrus, lingual gyrus, thalamus, cerebellum, midbrain PAG, and basal ganglia [37-41,49]. More specifically, the sensorimotor cortex region functionally includes the M1 and S1 and is anatomically located on/in the pre/postcentral gyrus in the frontal lobe and central sulcus [58]. The role of M1 is to generate neural impulses that control the execution of laryngeal movements [41]. Other regions of the cortex involved in motor function are called the secondary motor cortices. These regions include the premotor cortex, and the SMA and is anatomically located on/in the precentral gyrus and SFG, MFG, IFG [58]. The premotor cortex is involved in the sensory guidance of movement and adjusts the larynx before reaching for the phonation task. The SMA is involved in the planning and in coordinating of complex movements [56,59,60], such as vocal pitch modulation [176]. The SMA and the premotor regions both send information to the M1 as well as to brainstem motor regions. That is the main pathway for control of voluntary laryngeal movements in humans (Figure 4.1A). The midbrain PAG projects to the reticular formation of the lower brainstem, thus representing a neuroanatomical and functional relay station within the ACC-PAG-brainstem pathway (Figure 4.1A). The ACC and PAG guide the phonation for innate and emotional vocalization [61-65]. Moreover, activity of the cerebral cortex depends on impulses from the other modulatory brain regions. The cerebellum is involved in motor planning and coordination of laryngeal movements [66]. The lingual gyrus involved in simple phonemic tasks processing [67]. The MTG and STG are responsible for vocal self-monitoring [68] and voice processing [69], respectively. The insula participates in auditory vocal monitoring and detection, such as auditory attention and tuning in to novel auditory stimuli, temporal processing, and phonological processing [70] and integration of sounds with a speaker's emotions and attitudes [71]. Neural activity in the inferior parietal lobe reflects increased engagement of attentional resources [72]. Although our understanding of the neural correlates of non-disordered phonation in humans has increased significantly since the advent of neuroimaging, imaging studies of voice disorders are limited to a few specific voice pathologies such as spasmodic dysphonia [48-52], Parkinson's disease [53-55], and idiopathic unilateral vocal fold paralysis [56, 57]. This was the rationale to investigate the neural control of phonation in MTD patients.

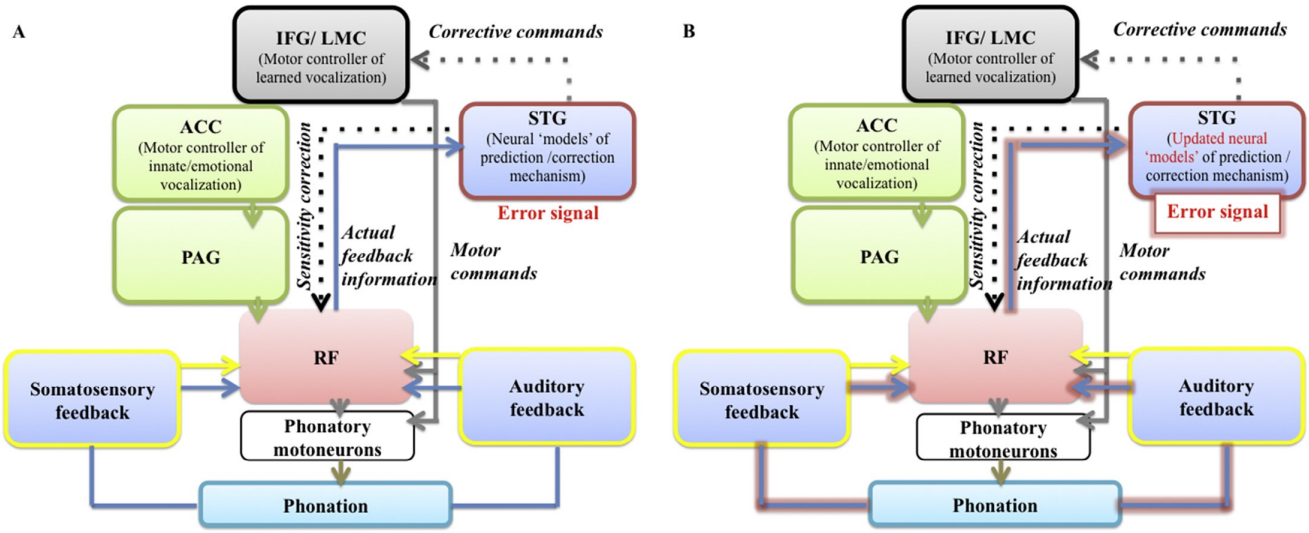


**Figure 4.1 A schematic diagram of laryngeal neural control of normal phonation (A) and phonation in muscle tension dysphonia (B) (modified from a neural model of vocalization proposed by Zarate [21]). A.** The vocal motor control system (central columns), reflexogenic system (yellow-outlined boxes and yellow arrows), and feedback system (blue boxes and arrows). The lower level of the vocal motor control system, the reticular formation (RF) (red box), generates complete vocal patterns to phonatory motoneurons (white box). The middle level of the motor control system, the anterior cingulate cortex (ACC) and periaqueductal gray (PAG) (green boxes), guides emotional vocalization. The upper level of the laryngeal motor control system, the laryngeal motor cortex (LMC), is responsible for producing learned/skilled vocalizations (ie, speech and song) and requires inputs from the inferior frontal gyrus (IFG) for motor planning of voice (other modulatory brain regions of the LMC are not depicted) (gray box). Feedback from phonation is processed by the ascending somatosensory (left) and auditory (right) pathways and transmitted to the superior temporal gyrus (STG) (blue boxes and arrows; the only selected regions of these pathways are shown) via the RF (red box). Sensory feedback from phonation provides actual information (how it feels), whereas the STG (red-outlined box; other possible brain regions involved in the prediction/correction mechanism are not depicted) provides information on the expected state (how should it feel) relying on a neural "model." The mismatch between actual sensory feedback and sensory predictions of motor com- mands indicates an error signal that, if large enough, would trigger changes in the neural model generating alterations in motor control (sending corrective commands [gray dotted arrow]) and sensory perception (changing sensitivity [black dotted arrow]). **B.** In MTD, the sensory stimulation associated with phonation is altered (indicated with red glowing arrows) and may trigger changes in the neural model: the mismatch between actual sensory information and prediction of the sensory outcome of motor commands (how should it feel) indicates an error signal (red glowing box). The error signal updates the neural model that in turn generates corrective commands to the motor controller as well as alter sensory perception. The updated or new neural model may support the symptoms of MTD by altering motor cortical commands in the areas responsible for motor control (eg, the LMC, IFG) and changing sensory perception (changes in sensitivity) in the areas responsible for sensory control (eg, the STG).

In this study, an fMRI evaluation of the neural control during phonation and exhalation was performed with a recently proposed protocol [73]. The experimental paradigm used consisted of sustained phonation of the sound /i/ on different pitch (habitual and high) levels and prolonged exhalation tasks [73]. The phonation tasks were designed to explore the interplay between respiratory and laryngeal control, whereas the exhalation tasks explored respiratory control separately. Additionally, the phonation tasks revealed the neural control associated with changes in respiratory and laryngeal adjustments to obtain vocal pitch modulations: comfortable and high. Comfortable phonation (i.e., habitual fundamental frequency [F0]) relies on a usual muscle tension (in as comfortable state as possible) in both the voicing and respiratory system. High phonation relies on a maximal/high muscular activity of the intrinsic and extrinsic laryngeal muscles and the respiratory system. In addition, this experimental paradigm allowed us to investigate laryngeal control maps that were generated by subtraction of the exhalation condition from the phonation condition. This approach is based on a study by Loucks et al [38], which showed that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation in healthy people, except for a difference in the STG activation due to the auditory feedback. These results were obtained during fMRI data analysis by subtracting patterns of neural control for voluntary exhalation from those during for phonation, considering the fact that if activity in a particular region of the brain during one task is greater than during another task, this particular region of the brain is involved in specific task-related activity [74-75].

The aims of this study were 1) to investigate brain activity during phonation in women with MTD in comparison with healthy controls; and 2) to explain the neurophysiological mechanism of laryngeal hyperfunction/tension during phonation in patients with MTD. The authors hypothesized that that compared with healthy controls, MTD patients may have altered brain activities related to phonation control. This altered brain activities of phonation control may be secondary to a peripheral sensory perturbations such as a poor vocal quality, upper respiratory infection, LPR, vocal demands, and/or life stress. Moreover, the authors hypothesized that the theory of the neural 'model' explains vocal hyperfunction during phonation in MTD patients.

## 2. Materials and methods

The study was performed as a prospective, interventional study. The Ethics Committee of Ghent University Hospital approved (B670201420193) the study protocol.

### Participants

Patients included in this study had a confirmed diagnosis of MTD by voice assessment protocol. The inclusion criteria for participants were as follows: (1) age between 21-45 years old, (2) female gender, (3) right-handedness, (4) being a native speaker of Flemish, (5) no organic laryngeal pathology (eg, nodules, polyps, laryngeal oedema), and (6) no history of neurological or psychiatric disease. The inclusion criteria for healthy subjects also were: absence of vocal pathology and videostrobolaryngoscopic symptoms of laryngeal pathology.

Ten patients (mean age: 33.2 years, age range: 21 – 47 years) and fifteen healthy subjects (mean age: 24.3 years, age range: 21 – 28 years) met the inclusion criteria and were recruited in the study. The rationale to include only adult healthy women was to reduce intra-group variance during fMRI data analysis. Healthy participants were recruited from the employees of Ghent University using an open advertisement. The patients with MTD were recruited at the Department of Otorhinolaryngology and Department of Speech, Language and Hearing Sciences at Ghent University Hospital, Belgium. Written informed consent was obtained from all participants.

### Questionnaires and voice handicap index

Prior to MRI scanning, all participants filled in a pre-scan MRI-safety questionnaire, the Edinburgh Handedness Inventory measurement scale, and a Personal History Questionnaire. These questionnaires were used to select participants who satisfy inclusion criteria, such as fMRI compatibility, medical history, lifestyle, and other participant characteristics. The psychosocial impact of vocal quality, as perceived by the subject, was measured by means of the validated Dutch translation of the VHI-10 [76]. This instrument assesses a subject's perception of disability, handicap, and distress resulting from voice

difficulties. It consists of 10 questions that cover emotional (2 questions), physical (3 questions), and functional (5 questions) aspects of the respondent's voice. The questions are rated on a 5-point ordinal scale: never (0), almost never (1), sometimes (2), almost always (3) and always (4). The total score ranges from 0 (no problem perceived) to 40. After scanning, participants completed a Post-Scan MRI-Checklist, which asks for information on the effects of the MRI equipment and its environment (i.e., magnetic field, acoustic noise).

## Clinical examination and voice assessment protocol

The same otorhinolaryngologist (S.C.) and speech therapist (E.D.) examined each subject clinically following a standard evaluation protocol. This protocol included a ENT and videostrobonasolaryngoscopic examination [77]. Clinical examination included focal palpation of tension around the larynx. The voice assessment protocol included a perceptual rating of the voice during connected speech by using the GRBASI scale and an objective vocal quality evaluation by means of the DSI [78]. The GRBASI scale consists of five well-defined parameters: G (overall grade of hoarseness), R (roughness), B (breathiness), A (asthenic), and S (strained) [79, 80]. A sixth parameter I for instability of the voice was added later to the original scale [81]. A four-point rating scale (0: normal, 1: slight, 2: moderate, and 3: severe) is used to indicate the grade of each parameter (Table 4.1). The objective parameters of the voice assessment protocol included the frequency range (F-low – F-high), the intensity range (I-low – I-high), aerodynamics (MPT and VC), and the acoustic microperturbations (jitter and shimmer) of voice during phonation of the vowel sound /a/ and /i/. The voice range was measured using the voice range profile module from the Computerized Speech Lab Model 4500 (CSL$^{TM}$, KayPENTAX, Lincoln Park, NJ). Recordings were made using a hand-held microphone (mouth-to-microphone distance = 7 cm). The acoustic analysis was performed with the Multi Dimensional Voice Program (MDVP) from the CSL. All measurements took place in a sound-treated room. Based on these results, the DSI was calculated using the following formula: $(0.13 \times \text{MPT}) + (0.0053 \times \text{F-high}) - (0.26 \times \text{I-low}) - (1.18 \times \text{Jitter}) + 12.4$ [78]. The DSI [78] is a multiparameter approach designed to establish an objective and quantitative correlate of the perceived vocal quality. The index ranges from −5 to +5 for severely dysphonic voices to normal voices. The more negative the index, the worse is the vocal quality. A DSI of 1.6 is the threshold separating normal voices

from dysphonic voices [83]. In addition, voice samples based on the production of sustained vowels /a/ and /i/ were used to determine the habitual F0 and the highest frequency (F-high) for each subject.

Subject selection was also based upon videostrobonasolaryngoscopic examination. The videostrobonasolaryngoscopy included phonation of the vowel sounds /a/ and /i/ at modal/comfortable, low-pitched, and high-pitched voice quality. The following videostrobonasolaryngoscopic indicators (at modal, low, and high pitch) were evaluated by the otorhinolaryngologist (S.C.) involved in our study: symmetry (symmetrical or asymmetrical), regularity (regular, irregular, or inconsistent), glottal closure (complete, incomplete, or inconsistent), type of gap (longitudinal, posterior, anterior, irregular, oval, or hour-glass), amplitude (increased, normal, reduced, or none), mucosal wave (normal, reduced, or none), and supraglottic activity [77]. Laryngeal supraglottic compression during videostrobonasolaryngoscopy was quantified by using the SERF protocol [84] by the otorhinolaryngologist (S.C.). The SERF form features a laryngeal image with concentric circles superimposed. Medio-lateral and anterior-posterior laryngeal constriction was evaluated separately by determining which numbered circle corresponds best to the observed degree of constriction (from 0: no constriction to 4: very severe constriction).

Diagnosis of MTD was based on following key features: (1) psychological and/or personality factors and stress influences [85, 86] and a history of vocal technical misuse/abuse and extraordinary voice demands [87-90] which were identified in the clinical history of patients; (2) a clinical sign of elevated extrinsic laryngeal muscle tension on palpation [91,92]; (3) voice assessment protocol with the DSI [78] (Table 4.1); and (4) features of MTD seen on videostrobonasolaryngoscopy [87] (Table 4.2). In MTD patients, the DSI range was from -13.2 to +2,5 (mean DSI = - 0,96) for phonation of the vowel sound /a/ and from -5.2 to 3.3 (mean DSI = 1.01) for phonation of the vowel sound /i/ (Table 4.1). In MTD patients, mean F0 of the vowel /i/ was 197,6 Hz (F0 range: 169 – 241,8 Hz) and mean F-high of the vowel /i/ was 528,9 Hz (F-high range: 311,1 – 680,3 Hz); mean F0 of the vowel /a/ was 193,4 Hz (F0 range: 164,2 – 232,7 Hz) and mean F-high of the vowel /a/ was 557,3 Hz (F-high range 329,6 – 932,3 Hz) (Table 4.1). Diagnosis of MTD on videostrobonasolaryngoscopy was established when one or more of following features were present: (1) open posterior commissure with a reduced amplitude and asymmetry of the mucosal waves; (2) a supraglottic contraction in which the ventricular folds are adducted to the midline; (3) an anteroposterior

contraction, which results in a foreshortening of the glottal aperture obscuring the posterior half to two-thirds of the vocal folds; or (4) complete anteroposterior contraction or squeeze of the supraglottis with approximation of the arytenoids to the petiole: "sphinteric larynx" [8, 93, 94]. The diagnosis agreement between the voice therapist and the laryngologist was made and calculated using percent agreement. Percent agreement is 71%. Based on the percent agreement between the voice therapist diagnosis of MTD and the laryngologist diagnosis of MTD, 10 patients were included in the study and 4 patients were excluded from the study because of disagreements.

Each healthy subject had unchanged measures of a voice assessment protocol and a DSI value corresponding to a normal voice quality[202] (mean DSI of the vowel /a/: + 3.9, DSI range +1.7 – +6.2; mean DSI of the vowel /i/: +3.8, DSI range +1.2 – +7.4) (Table 4.1). In healthy participants, mean F0 of the vowel /i/ was 211 Hz (F0 range: 172,5 – 229,3 Hz) and mean F-high of the vowel /i/ was 799,3 Hz (F-high range: 622,3 – 1046,5 Hz); mean F0 of the vowel /a/ was 199,5 Hz (F0 range: 161,2 – 217,7 Hz) and mean F-high of the vowel /a/ was 848,6 Hz (F-high range: 622,3 – 1174,7 Hz) (Table 4.1). Videostrobonasolaryngoscopic evaluations of the healthy participants showed normal laryngeal structure and function during phonation of /i/ and /a/ at modal/comfortable, low-pitched and high-pitched voice quality (Table 4.2).

## Functional magnetic resonance imaging experimental protocol

The fMRI experiment was performed with the recently proposed protocol [73]. A blocked design fMRI experiment consisted of multiple epochs of stimulation lasting 14.5 seconds followed by a period of rest ranging between 11 and 20 seconds (variable jittering). Jittered inter-stimulus intervals – rest periods – were used to better determine the shape of whole hemodynamic responses function (HRF) and to find a good baseline to evaluate response peaks [95]. The block of maximum 34.5 seconds was repeated 12 times for each condition. Each experimental condition had a total duration of 414 seconds. All participants were tested under three different conditions, which were randomized in the different order for each participant. These conditions were: (1) COMFORTABLE PHONATION: prolonged phonation of a vowel /i/ (similar to the "ee" in "see") on a habitual pitch level; (2) HIGH-PITCHED PHONATION: prolonged phonation of

the same vowel /i/ using a high voice pitch; and (3) PROLONGED EXHALATION: voluntary sustained "unvoiced" oral exhalation.

| **Feature** | **Healthy group (n=15)** | | **MTD group (n=10)** | |
|---|---|---|---|---|
| **Vocal assessment protocol** | **Mean (Standard deviation (SD)) sustained vowel /a/** | **Mean (SD) sustained vowel /i/** | **Mean (SD) sustained vowel /a/** | **Mean (SD) sustained vowel /i/** |
| **Vocal range** | | | | |
| Lowest intensity (dB) | 53.5 (2,5) | 54,1 (3) | 59 (3,3) | 56,7 (2,3) |
| Highest intensity (dB) | 101,4 (4,4) | 94,3 (3,7) | 94,2 (7,7) | 92 (6,8) |
| Lowest frequency (Hz) | 132,9 (20) | 124,9 (45,6) | 142,6 (29,1) | 137,5 (25) |
| Highest frequency (Hz) | 848,6 (166,7) | 799,3 (137) | 557,3 (202) | 528,9 (111,5) |
| Fundamental frequency F0 (Hz) | 199,5 (17,4) | 211,6 (16) | 193,4 (34,9) | 197,6 (24,3) |
| **Aerodynamics** | | | | |
| Maximum phonation time [194] | 19,7 (4,9) | 22,6 (5,1) | 12,29 (7) | 21 (6,8) |
| Vital capacity ($cm^3$) | 2630 (478,8) | 2610 (520) | 2475 (560) | 2425 (462,6) |
| **Acoustic analysis** | | | | |
| Jitter (%) | 1,5 (0,7) | 1,6 (1) | 3,2 (2,5) | 2,2 (1) |
| Shimmer (%) | 5,3 (1,7) | 2,7 (0,9) | 7,9 (5,8) | 4,3 (3,5) |
| **DSI** | 3,9 (1,3) | 3,8 (2,0) | -0,96 (4,7) | 1,01 (2,4) |
| **VHI-10** | **Mean** | **SD** | **Mean** | **SD** |
| VHI functional | 2,6 | 2,1 | 7,2 | 6,9 |
| VHI physical | 1,9 | 2,3 | 11,7 | 8,1 |
| VHI emotional | 0,6 | 1 | 5,2 | 8,4 |
| VHI total (0-40) | 5,1 | 4,2 | 24,1 | 22,9 |
| **GRBASI** | **Mean** | **SD** | **Mean** | **SD** |
| **G** | 0 | 0 | 0.9 | 1.1 |
| **R** | 0 | 0 | 0.7 | 0.8 |
| **B** | 0 | 0 | 1.1 | 0.9 |
| **A** | 0 | 0 | 0.9 | 1.1 |
| **S** | 0 | 0 | 0.4 | 1.1 |
| **I** | 0 | 0 | 0.3 | 0.8 |

**Table 4.1: Voice Assessment Protocol, Voice Handicap Index (VHI) and GRBASI in healthy women and women with MTD**

| Videostroboscopic Feature | Healthy group | | MTD group | |
|---|---|---|---|---|
| | n | % | n | % |
| **Symmetry** | | | | |
| Symmetrical | 15 | 100 | 4 | 40 |
| Asymmetrical | - | - | 6 | 60 |
| **Regularity** | | | | |
| Regular | 15 | 100 | 4 | 40 |
| Irregular | - | - | 6 | 60 |
| Inconsistent | - | - | | |
| **Glottic closure** | | | | |
| Complete | 14 | 93,3 | 2 | 20 |
| Incomplete | 1 | 6,7 | 8 | 80 |
| **Type glottal gap** | - | - | | |
| Longitudinal | - | - | 5 | 50 |
| Posterior | 1 | 6,7 | 3 | 30 |
| Anterior | - | - | 1 | 10 |
| Oval | - | - | - | - |
| Hour-glass | - | - | 1 | 10 |
| **Amplitude** | | | | |
| Normal | 13 | 86,7 | 3 | 30 |
| Reduced | 2 | 13,3 | 7 | 70 |
| Increased | - | - | - | - |
| **Mucosal wave** | | | | |
| Normal | 16 | 100 | 3 | 30 |
| Reduced | - | - | 7 | 70 |
| None | - | - | - | - |
| **A-P constriction** | | | | |
| 0 | 13 | 86,7 | 1 | 10 |
| 1 | 2 | 13,3 | 2 | 20 |
| 2 | - | - | 4 | 40 |
| 3 | - | - | 3 | 30 |
| 4 | - | - | - | - |
| **M-L constriction** | | | | |
| 0 | 14 | 93,3 | 2 | 20 |
| 1 | 1 | 6,7 | 3 | 30 |
| 2 | - | - | 2 | 20 |
| 3 | - | - | 3 | 30 |
| 4 | - | - | - | - |

**Table 4.2: Videostroboscopic features in healthy women and women with MTD.** Abbreviations: A-P, anterior-posterior constriction (0 no constriction, 4 severe constriction); M-L, medio-lateral constriction (0 no constriction, 4 severe constriction).

Periods during which the volunteers had to perform a task were visually indicated during 10 seconds by a grey loading bar, whereas resting periods were indicated by a black cross. Two visual instructions between the actual tasks were presented in the subject's native language indicating the type of task (2 seconds) (i.e. in Dutch: "Gewone Stem", "Hoge Stem" or "Verlengde Uitademing") and a visual cue to start inspiration (2.5 seconds) (i.e. in Dutch: "Inademen"). All visual commands were generated

using a commercially available experiment generator (Presentation, Neurobehavioral Systems Inc., Berkeley, CA, USA) and were reflected in a mirror on the head coil.

Prior to scanning, speech pathologist explained to all participants how to produce a sustained vowel /i/ during 10 seconds using a comfortable as well as a high pitch and to sustain exhalation for the same duration for the fMRI study. Objective measures of the vocal quality during task production were not used, as these measures were not implemented in the fMRI experiment.

### *Magnetic resonance imaging acquisition*

FMRI images were acquired on a 3-Tesla MR scanner (Siemens Magnetom Trio, Erlangen, Germany) using the standard 32-channel head coil. Initially, an anatomical T1-weighted MR dataset covering the whole head at 1 $mm^3$ isotropic resolution was acquired (high-quality three-dimensional magnetization-prepared rapid acquisition with gradient echo (3D MPRAGE) images, repetition time = 1950 ms, inversion time =1100 ms, echo time = 3.93 ms, flip angle = 12°). An axial T2*-sensitive gradient-echo echo-planar imaging technique with an in-plane resolution of $2x2mm^2$ was used to generate the functional images (repetition time = 2000 ms, echo time = 36 ms, flip angle = 70°, acquisition matrix = 96x128). Forty consecutive sections of 3-mm thickness with 0.5 mm gap between slices in an axial-to-coronal orientation were acquired. A total of 176 volumes were recorded for experimental run, resulting in a total investigation time of 25 minutes.

Subjects were positioned head-first and supine inside the magnet bore and fitted with a OptoACTIVE noise cancelling MRI headphone and a FOMRI-III noise cancelling microphone (OptoActive™, Optoacoustics Ltd, Moshav Mazor, Israel). The OptoACTIVE system provided a high level of noise reduction and self-monitoring of voice during phonation. Each participant's head was immobilized in the standard head coil using neck cushions to minimize motion artifacts. The subjects were instructed to keep their jaw, lips and tongue motionless while performing the tasks and to keep their jaw slightly open in order to minimize movements during phonation (e.g. movements of orofacial muscles), which might also cause artifacts during fMRI scanning. In addition, participants reduced articulatory gestures due to sustained phonation of the vowel /i/ at a constant pitch during phonation tasks. The project leader (S.C.)

and MRI operator (M.K.) monitored the performance of tasks throughout the experiment through a control room speaker to insure that each participant correctly performed the phonation tasks.

### *Image analysis steps*

All steps of fMRI data preprocessing and fMRI data analysis (intragroup and intergroup) were performed using the BrainVoyager QX Version 2.4 software package (Brain Innovation, Maastricht, The Netherlands) [44]. Preprocessing included 3D motion correction, and slice timing correction and normalization to a standard echo planar imaging (EPI) template based on neuroanatomical atlas of Talairach and Tournoux [96]. Finally, normalized images were spatially smoothed on volume time course (VTC) files with a Gaussian kernel for the full width at the half maximum (FWHM) of 8 mm (the voxel size of resultant VTC was $3 \times 3 \times 3$ $mm^3$). A statistical parametric map was calculated using the approach of the general linear model (GLM). For each experiment, a BrainVoyager protocol file (PRT) was derived, representing the onset and duration of the events for the different conditions and rest period as a baseline. From the created protocols, the design matrices for the calculation of the GLM were defined automatically. In order to account for hemodynamic response, each of the predictors was derived by convolution of the block design with a model for the two gamma hemodynamic response functions [75]. Previously, the GLM design matrix was improved by defining proper noise predictors using the ICA approach [97]. After fitting the GLM [98], group t-maps were generated by invoking the RFX-ANCOVA (Analysis of Covariance-Random Effect Analysis) tool and using a subtraction approach [38, 74, 75] for fMRI data analysis of the comfortable phonation, high-pitched phonation and prolonged exhalation as well as for the comparisons between conditions of phonation and prolonged exhalation. Activation maps were generated by thresholding the statistical maps using $P<.001$, 10 voxels, uncorrected [99].

Comparison of two groups (MTD vs healthy) was performed using a "combine maps" approach ($P<.005$, 10 voxels, uncorrected). First, the separate maps for the different subjects (VTC for 25 subjects in total) and for the contrasts/conditions chosen in every subject were created. Second, the different maps were separated into different groups (G1 and G2), which enabled specific statistics on the basis of the maps separated into groups. Then the T-test (G1 vs G2) to compare the activation pattern found in the groups was

used. All subjects in G1 – MTD group and G2 – healthy group were selected. BrainVoyager automatically created a new map into Overlay Maps dialog that contained the result for the specified conditions: comfortable, high-pitched phonation, and prolonged exhalation. The neuroimaging activation maps were checked to display the results in the VMR dataset. Comparison of two groups was performed using a subtraction approach [38, 74, 75] for fMRI data analysis of the comfortable phonation, high-pitched phonation and prolonged exhalation as well as for the comparisons between conditions of phonation and prolonged exhalation.

## 3. Results

There were no significant group differences at our initial FDR-corrected threshold. However, exploratory analyses at a lowered threshold ($p < .001$ 10 voxels, uncorrected) have revealed significant activation in the brain. The data analysis has shown that areas of activation in the MTD and control groups resembled those in other fMRI studies on phonation involving simple voice production tasks in healthy people [37, 38, 40, 42, 47, 49]. Brain activation during phonation was observed in the bilateral precentral gyrus, right SFG, MFG and IFG, lingual gyrus, cingulate gyrus, STG, thalamus (ventral posterior lateral nucleus), and bilateral cerebellum in the two groups (Table 4.3). Statistical analysis also identified a significant effect of exhalation ($P<.001$, 10 voxels, uncorrected) in the bilateral precentral gyrus, cingulate gyrus, right lingual gyrus, and bilateral cerebellum in both groups (Table 4.4) which is corroborated by recent fMRI study by Loucks et al [38].

Comparison of phonation (comfortable, high-pitched) tasks with prolonged exhalation tasks identified activation in the bilateral STG and insula in the two groups (Table 4.4). However, the fMRI data analysis for the high-pitched phonation compared to comfortable phonation did not reveal any significant activation in the brain in the two groups.

| Area | Healthy Group (n=15) | | | | MTD Group (n=10) | | | |
|---|---|---|---|---|---|---|---|---|
| | Brodmann No | Cluster size ($mm^3$) | t(42) (peak) | Talairach coordinates x,y,z | Brodmann No | Cluster size ($mm^3$) | t(27) (peak) | Talairach coordinates x,y,z |
| **COMFORTABLE PHONATION** | | | | | | | | |
| Right precentral gyrus | 3,4,6 | 1671 | 5,7 | 46;-5;47 | 3,4,6 | 228 | 4,6 | 49;-6;42 |
| Left precentral gyrus | 3,4 | 1509 | 6,3 | -43;-18;35 | 3,4 | 1324 | 5,0 | -43;-16;34 |
| Right middle frontal gyrus | 10 | 53 | 3,5 | 37;40;6 | 46 | 494 | 3,9 | 44;40;-6 |
| Right inferior frontal gyrus | 9 | 414 | 4,5 | 52;7;30 | 9 | 303 | 6,4 | 52;7;30 |
| Cingulate gyrus | 31 | 912 | 5,1 | -3;-59;27 | 31 | 85 | 4,0 | -7;-51;25 |
| Right lingual gyrus | 18 | 540 | 4,5 | 28;-84;-2 | 18 | 369 | 4,7 | 32;-75;-7 |
| Right superior temporal gyrus | 22,41,42 | 1192 | 5,0 | 61;-30;9 | 41 | 240 | 4,7 | 60;-24;13 |
| Thalamus (ventral posterior lateral nucleus) | | 120 | 4,2 | 10;-14;19 | | 59 | 3,7 | -6;-30;15 |
| Right Cerebellum | | 107 | 2,9 | 21;-53;-26 | | 77 | 4,6 | 23;-53;-20 |
| Left Cerebellum | | 270 | 3,8 | -29;-55;-26 | | 336 | 5,0 | -19;-56;-24 |
| **HIGH-PITCHED PHONATION** | | | | | | | | |
| Right precentral gyrus | 4,6 | 3028 | 6,1 | 40;-10;36 | 4 | 164 | 4,8 | 48;-6;42 |
| Left precentral gyrus | 3,4,6 | 916 | 5,3 | -40;-15;38 | 2-4,6 | 1318 | 6 | -36;-14;32 |
| Right superior frontal gyrus | 6,8 | 321 | 5,2 | 23;22;50 | 8 | 381 | 5 | 22;17;45 |
| Right inferior frontal gyrus | 9 | 1095 | 5,4 | 51;7;31 | 9 | 134 | 5,6 | 51;7;31 |
| Right middle frontal gyrus | 6,9 | 627 | 4,8 | 35;25;31 | 9 | 64 | 3 | 46;31;31 |
| Cingulate gyrus | 31 | 400 | 4,4 | -9;-52;25 | 31 | 150 | 4 | -9;-52;25 |
| Left lingual gyrus | | | | | 18 | 535 | 5 | -10;-77;-7 |
| Right superior temporal gyrus and insula | 22,41,42 | 2234 | 5,5 | 61;-29;9/52;-7;4 | 13, 41,42 | 198 | 4.7 | 58;-32;14/41;5;21 |
| Left superior temporal gyrus, insula | 22 | 446 | 5,8 | -47;-13;-2 | 22 | 75 | 4,7 | -43;-22;0 |
| Right thalamus (ventral posterior lateral nucleus) | | 566 | 4,0 | 13;-10;21/-13;-10;21 | | 215 | 3,6 | 17;-10;13/-26;-18;1 |
| Right cerebellum | | 528 | 5,5 | 3;-75;-28 | | 49 | 4,4 | 23;-54;-21 |
| Left cerebellum | | 333 | 5,1 | -34;-54;-28 | | 268 | 4,6 | -48;-56;-39 |

**Table 4.3: Brain activation during phonation in the healthy and MTD groups.** Regions of significant activation are listed for each condition and for relevant contrasts between the conditions. Results are presented in Talairach space ($p<.001$, uncorrected).

| Area | Healthy Group (n=15) | | | | MTD Group (n=10) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Brodmann No** | **Cluster size ($mm^3$)** | **t(42) (peak)** | **Talairach coordinates x,y,z** | **Brodmann No** | **Cluster size ($mm^3$)** | **t(27) (peak)** | **Talairach coordinates x,y,z** |
| **PROLONGED EXHALATION** | | | | | | | | |
| Right Precentral Gyrus | 3,4,6 | 1332 | 4,7 | 46;-10;35/48;3;19 | 3,4,6 | 195 | 4 | 46;-10;-35/36;-17;36 |
| Left Precentral Gyrus | 3,4,6 | 933 | 4,4 | -46;-16;34 | 3,4,6 | 755 | 4,5 | -41;-18;36 |
| Cingulate gyrus | 31,32 | 1042 | 3,4 | 22;28;19 | 31,32 | 311 | 4,4 | 22;-9;32 |
| Right Lingual Gyrus | 18,19 | 2621 | 5,1 | 31;-79;-3 | 18,19 | 605 | 4,3 | 28;-84;-2 |
| Right Cerebellum | | 567 | 3,2 | 19;-57;-23 | | 88 | 3,2 | 32;-42;-28 |
| Left Cerebellum | | 166 | 2,8 | -17;-60;-21 | | 240 | 3,4 | -28;-68;-23 |
| **COMFORTABLE PHONATION>PROLONGED EXHALATION** | | | | | | | | |
| Right superior temporal gyrus and Insula | 13,21,22,41 | 2598 | 5,1 | 52;-24;9 | 13,22,41,42 | 1214 | 6,7 | 59;-29;8/41;-25;8 |
| Left superior temporal gyrus and Insula | 13,21,22,41 | 975 | 5,9 | -47;-24;6 | 22,41 | 2447 | 5,1 | -45;-26;7 |
| **HIGH-PITCHED PHONATION>PROLONGED EXHALATION** | | | | | | | | |
| Right superior temporal gyrus and Insula | 13,21,22,41 | 5006 | 5,5 | 50;-20;7 | 13,21,22,41,42 | 245 | 5,5 | 40;-27;8 |
| Left superior temporal gyrus and Insula | 13,21,22,41 | 2876 | 6,9 | -46;-21;4 | 13,21,22 | 2328 | 5,5 | -46;-23;3 |

**Table 4.4: Brain activation during phonation and exhalation in the healthy and MTD groups.** Regions of significant activation are listed for each condition and for relevant contrasts between the conditions. Results are presented in Talairach space ($p<.001$, uncorrected).

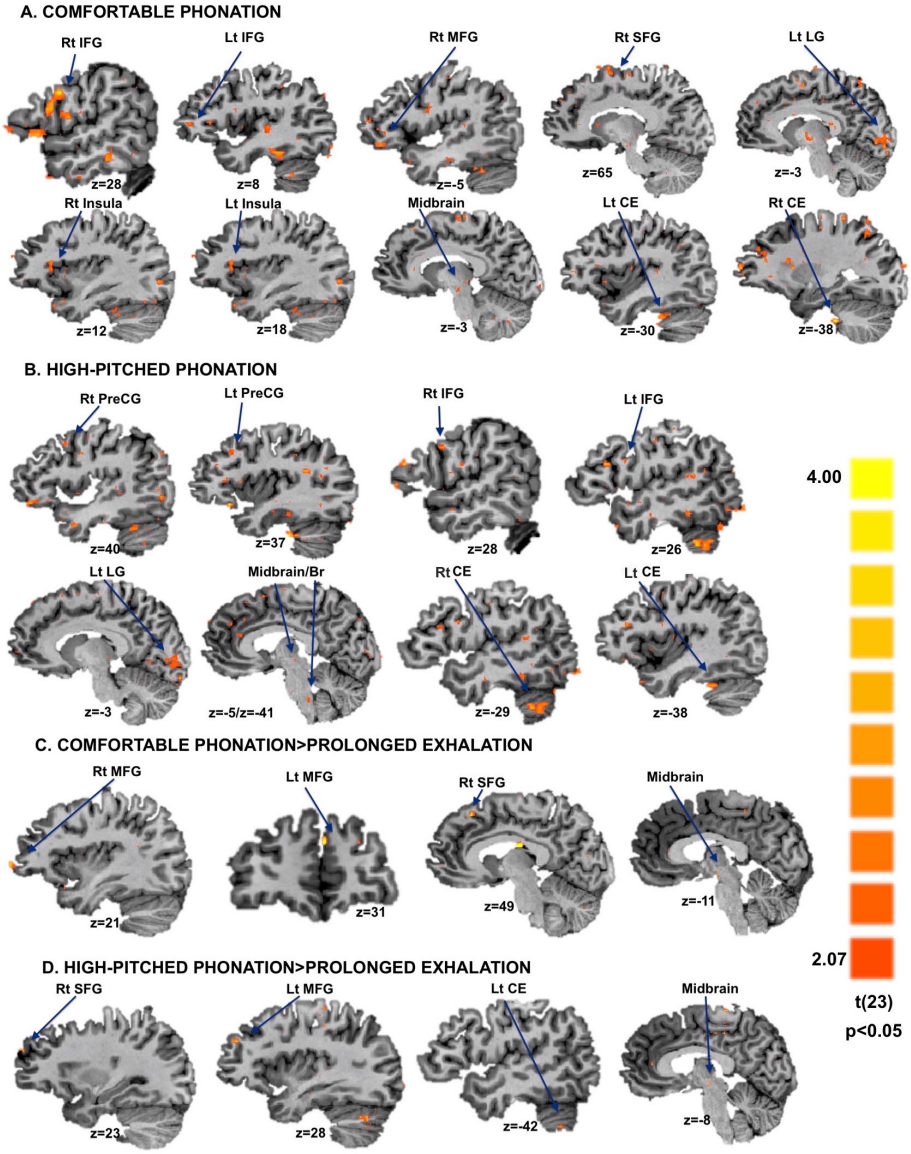


**Figure 4.2: Areas of higher brain activation (p<.005, 10 voxels, uncorrected) during phonation in patients with MTD compared with controls for the conditions of COMFORTABLE PHONATION (A), HIGH-PITCHED PHONATION (B), COMFORTABLE PHONATION>PROLONGED EXHALATION (C), and HIGH-PITCHED PHONATION>PROLONGED EXHALATION (D).** For fuller visualization of cluster extent, results are illustrated at a threshold of p<.05 (uncorrected), and an extent threshold of 10 contiguous voxels. The arrows indicate clusters of significant activation (p<.005, uncorrected), z coordinates are given below each slice (Abbreviations: Rt: right; Lt: left; IFG: inferior frontal gyrus; MFG: middle frontal gyrus: SFG: superior frontal gyrus; LG: lingual gyrus; CE: cerebellum; PreCG: precentral gyrus; Br: brainstem).

Comparison of phonation tasks ($p<.005$, 10 voxels, uncorrected) in the two groups (MTD vs healthy) revealed *higher* brain activities during phonation (comfortable pitch, high-pitched) in the precentral gyrus, inferior, middle and superior frontal gyrus, lingual gyrus, insula, cerebellum, midbrain, and brainstem – laryngeal motor control-related areas – in the MTD group (Table 4.5, Figure 4.2). Areas with *lower* activation during phonation (comfortable, high-pitched) were observed in the cingulate gyrus, MTG and STG, and inferior parietal lobe in the MTD group in comparison with healthy controls (Table 4.6, Figure 4.3). No differences were found between two groups regarding exhalation control. Comparison of prolonged exhalation tasks in the two groups (MTD vs healthy) indicated a completely overlapping pattern of responses in the cerebral regions mentioned above (Table 4.4). Furthermore, comparison of phonation (comfortable and high-pitched) tasks with prolonged exhalation tasks in the two groups (MTD vs healthy) revealed areas with *higher* activation in the middle and superior frontal gyrus, and midbrain in the MTD group (Table 4.5, Figure 4.2) and areas with *lower* activation in the left middle temporal gyrus for comfortable phonation and in the right inferior parietal lobe for high-pitched phonation in the MTD group (Table 4.6, Figure 4.3).

| Area | Brodmann No | Cluster size ($mm^3$) | t(23) (peak) | Talairach coordinates x,y,z |
|---|---|---|---|---|
| **COMFORTABLE PHONATION** | | | | |
| Right Inferior Frontal Gyrus | 9 | 628 | 4,5 | 53;9;28 |
| Left Inferior Frontal Gyrus | 9,46 | 179 | 4,4 | -45;2;21/-43;42;8 |
| Right Middle Frontal Gyrus | 47 | 205 | 3,1 | 42;40;-5 |
| Right Superior Frontal Gyrus | 6 | 191 | 3,5 | 7;6;65 |
| Left Lingual Gyrus | 17 | 579 | 4 | -13;-87;-3 |
| Right Insula | 13 | 194 | 3,7 | 31;25;12 |
| Left Insula | 13 | 534 | 4,5 | -31, 9, 18 |
| Right Cerebellum | | 436 | 3,6 | 36;-36;-30 |
| Left Cerebellum | | 224 | 4,2 | -28;-30;-38 |
| Midbrain PAG | | 94 | 3,1 | 4;-24;-3 |
| **HIGH-PITCHED PHONATION** | | | | |
| Right Precentral Gyrus | 6 | 83 | 4,0 | 40;14;40 |
| Left Precentral Gyrus | 9 | 76 | 2,7 | -40;21;37 |
| Right Inferior Frontal Gyrus | 9 | 73 | 3,2 | 53;9;28 |
| Left Inferior Frontal Gyrus | 9 | 47 | 3,0 | -49;7;26 |
| Left Lingual Gyrus | 17 | 522 | 2,9 | -14;-87;-3 |
| Right Cerebellum | | 364 | 3,4 | 37;-37;-29 |
| Left Cerebellum | | 1248 | 4,0 | -48;-56;-38 |
| Midbrain PAG/Brainstem | | 17/65 | 2,6/3,1 | 0;-21;-5/0;-32;-41 |
| **COMFORTABLE PHONATION>PROLONGED EXHALATION** | | | | |
| Right Middle Frontal Gyrus | 8,10 | 128 | 4,0 | 34;38;21 |
| Left Middle Frontal Gyrus | 9 | 20 | 4,3 | -7;46;31 |
| Right Superior Frontal Gyrus | 8 | 30 | 4,1 | 2;28;49 |
| Midbrain PAG | | 25 | 4,2 | -2;-14;-11 |
| **HIGH-PITCHED PHONATION>PROLONGED EXHALATION** | | | | |
| Right Superior Frontal Gyrus | 10 | 25 | 3,6 | 26;59;23 |
| Left Middle Frontal Gyrus | 9 | 46 | 3,9 | -37;44;28 |
| Midbrain PAG | | 17 | 4,3 | -1;-19;-8 |
| Left Cerebellum | | 53 | 4,7 | -48;-56;-42 |

**Table 4.5. Areas with higher activation in the MTD group compared with the control group.** Results are presented in Talairach space ($p<.005$, uncorrected).

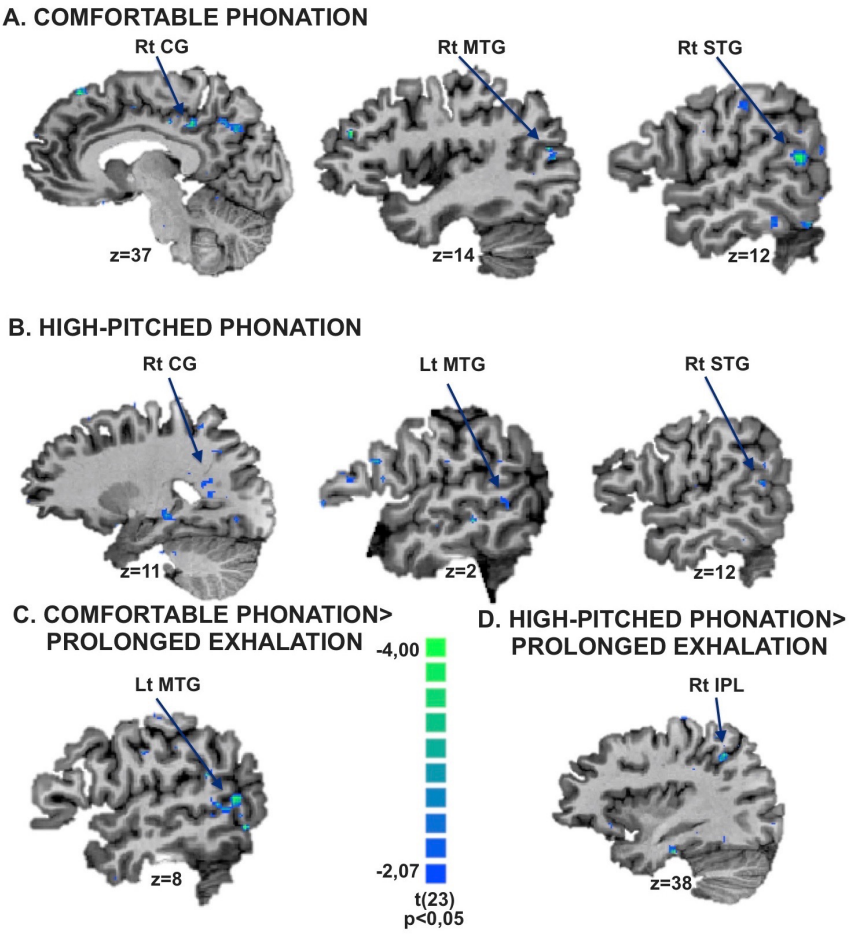


**Figure 4.3 Areas of lower brain activation (p<.005, 10 voxels, uncorrected) during phonation in patients with MTD compared with controls for the conditions of COMFORTABLE PHONATION (A), HIGH-PITCHED PHONATION (B), COMFORTABLE PHONATION>PROLONGED EXHALATION (C), and HIGH-PITCHED PHONATION>PROLONGED EXHALATION (D).** For fuller visualization of cluster extent, results are illustrated at a threshold of p<.05 (uncorrected), and an extent threshold of 10 contiguous voxels. The arrows indicate clusters of significant activation, z coordinates are given below each slice. (Abbreviations: Rt: right; Lt: left; CG: cingulate gyrus; MTG: middle temporal gyrus; STG: superior temporal gyrus; IPL: inferior parietal lobe).

| Area | Brodmann No | Cluster size ($mm^3$) | t(42) (peak) | Talairach coordinates x,y,z |
|---|---|---|---|---|
| **COMFORTABLE PHONATION** | | | | |
| Right Cingulate Gyrus | 31 | 1066 | -5 | 2;-34;37 |
| Right Middle Temporal Gyrus | 39 | 67 | -3,0 | 41;-64;15 |
| Left Middle Temporal Gyrus | 39 | 570 | -3,5 | -41;-74;14 |
| Right Superior Temporal Gyrus | 22 | 491 | -4,7 | 52;-49;12 |
| **HIGH-PITCHED PHONATION** | | | | |
| Cingulate Gyrus | 31 | 468 | -3 | 19;-53;11 |
| Left Middle Temporal Gyrus | 21 | 75 | -3 | -55;-53;2 |
| Right Superior Temporal Gyrus | 21, 22 | 302 | -3,8 | 51; -47; 12 |
| **COMFORTABLE PHONATION>PROLONGED EXHALATION** | | | | |
| Left Middle Temporal Gyrus | 39 | 54 | -4,3 | -52;-62;8 |
| **HIGH-PITCHED PHONATION>PROLONGED EXHALATION** | | | | |
| Right Inferior Parietal Lobe | 40 | 61 | -4,5 | 31;-48;38 |

**Table 4.6 Areas with lower activation in the MTD group compared with the control group.** Results are presented in Talairach space ($p<.005$, uncorrected).

## 4. Discussion

The neurophysiological mechanisms of how brain controls phonation are practically unknown. The purposes of this study were 1) to detect brain activity during phonation in women with MTD in comparison with healthy controls; and 2) to explain the neurophysiological mechanism of laryngeal hyperfunction/tension during phonation in patients with MTD. We hypothesized that MTD patients have altered brain activities of phonation control secondary to a peripheral sensory perturbations such as a poor vocal quality, upper respiratory infection, LPR, vocal demands, and/or life stress. Moreover, the authors hypothesized that the theory of the neural 'model' explains vocal hyperfunction during phonation in MTD patients.

Ten women with MTD and fifteen healthy women participated in the study. We implemented an experimental paradigm consisting of sustained phonation of /i/ and prolonged exhalation tasks. The phonation tasks explored both respiratory and laryngeal control as well as the neural control associated with pitch (comfortable and high) modulations. The exhalation tasks explored respiratory control and allowed to

generate laryngeal control maps by subtraction of the exhalation condition from the phonation condition [38, 74, 75].

In our study, brain activity in response to phonation of sound /i/ in related vocal pitch (comfortable and high) changes was observed in the bilateral precentral gyrus, right SFG, MFG and IFG, lingual gyrus, cingulate gyrus, STG, thalamus (ventral posterior lateral nucleus), and bilateral cerebellum in the two (MTD and healthy) groups (Table 4.3). These results are corroborated by recent fMRI studies on phonation involving simple voice production tasks [37, 38, 41, 42, 47, 49]. The previously reviewed studies have observed activity in the same auditory, motor, frontal, parietal, and subcortical brain areas during phonation that are specialized for different functions. More specifically, bilateral activations in the precentral gyrus, the MFG and the IFG are related to laryngeal motor control areas [41]. The MTG and the STG are responsible for vocal self-monitoring [68] and sensory voice processing and/or sensorimotor integration for vocal production [69, 100], respectively. Cingulate cortex activity is associated with volitional motor control necessary for phonation, especially during pitch [100] and emotional vocal modulations [102]. Activation in the cerebellum is involved in motor planning and coordination of laryngeal movements [66]. The lingual gyrus activity involved in simple phonemic tasks processing obviating the need for more efforts for the task [67]. Additionally, in our experiment, activity in the bilateral SFG was present during the high-pitch phonation task only in the two groups. Goldberg et al [103] found that when a personal emotional response was required, participants showed activity in the SFG – the brain region associated with self-awareness-related function. In our experiment, activity in the bilateral SFG was present during the high pitch-phonation task, hypothetically reflecting greater emotional activity co-occurring with higher vocal effort required to control high-pitched phonation.

Additionally, to test whether sensory input affects brain activity during vocal pitch modulation, a comparison between comfortable pitch and high-pitch phonation in MTD and control groups was performed. Since pitch modulation is based on modifying laryngeal and respiratory control [104] – where both auditory and somatosensory inputs are different – we expected different brain activities. However, these tasks were unable to show brain activation difference between high-pitched and comfortable phonation in MTD patients and control subjects. In our study, an experimental paradigm involving phonation of the /i/ sound was used in order to avoid major resonance articulatory changes as used in the fMRI studies by

Loucks et al [38], Haslinger et al [49] and Simonyan and Ludlow [48]. However, in order to reduce articulatory modifications during phonation, subjects performed phonation of sound /i/ with reduced labial and jaw movements rather than natural phonation tasks. For future research, in order to explore vocal pitch modulation control, an experimental paradigm with phonation of the vowel /a/ instead of /i/ sound may be recommended to avoid F0 coinciding with the first resonance [105].

The exhalation tasks in the present study explored respiratory control in MTD and control groups. Statistical analysis identified a significant effect of exhalation in the bilateral precentral gyrus, cingulate gyrus, right lingual gyrus, and bilateral cerebellum in the both groups (Table 4.4). In the fMRI study by Loucks et al [38], a comparable pattern of responses was identified for exhalation control in healthy subjects involving the left ventrolateral cortex, precentral and postcentral gyri, right supramarginal gyrus, right lingual gyrus, right cerebellum and thalamus. In addition, the exhalation task allowed to generate laryngeal sensorimotor control maps by subtraction of the exhalation condition from the phonation condition [38, 74, 75]. Since the single cluster of differential activation in SFG was the only difference for the comfortable phonation and high-pitched phonation, these conditions were combined when comparing phonation (comfortable and high) and prolonged exhalation. This comparison revealed brain activity in the bilateral STG and insulla – the brain regions associated with sound perception – in the two groups (Table 4.4).

The group comparison of prolonged exhalation tasks in patients with MTD versus healthy controls has determined overlapping pattern of responses in the cerebral regions typically active during normal exhalation. It showed that the neural control of exhalation, specifically of exhalation for phonation in patients with MTD, is not altered [38]. This assumption is based on the conclusion of fMRI study by Loucks et al [38], which showed that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation. Only a difference in STG activation was seen, due to the auditory feedback. These results were obtained by subtracting neural control of voluntary exhalation from neural control of phonation during fMRI data analysis in order to focus on sensory feedback control of phonation. Furthermore, no difference between the two groups in the exhalation tasks allowed a comparison of these tasks with the phonation tasks to identify the regions that are involved in sensory feedback control of phonation.

The imaging results supported our hypothesis that patients with MTD, when compared to healthy subjects, may have altered brain activity related to phonation control. Compared with controls, during phonation, MTD patients showed *higher* activation in the laryngeal motor control-related areas such as the precentral gyrus SFG, MFG, insula, midbrain, brainstem and cerebellum. Furthermore, comparison of phonation (comfortable and high-pitched) tasks with prolonged exhalation tasks identified areas with *higher* activation in the MFG and SFG in the MTD group versus control. Thus, the brain response observed in the present study may reflect that MTD patients control their voice by use of the laryngeal motor control-related areas, midbrain, brainstem and cerebellum. Lower neural activation was seen in the cingulate gyrus, STG and MTG and inferior parietal lobe in the MTD group in comparison with healthy controls. Moreover, comparison of phonation (comfortable, high-pitched) with prolonged exhalation tasks identified areas with *lower* activation in the left MTG for comfortable phonation and in the right inferior parietal lobe for high-pitched phonation in the MTD group in comparison with healthy controls. Since scanner noise was minimized during scanning, the subject's own voice served as the auditory stimulus and was taken to reflect auditory cortex activation.

In patients with MTD, these altered (higher/lower) brain activities may result in laryngeal tension and voice symptoms. However, this experiment did not provide evidence of internal representations/neural 'model' of the sensorimotor transformations changes. This experiment did, however, provide evidence of altered neural correlates of phonation in MTD. In our study, altered neural activities were presented during phonation in MTD patients in comparison with healthy controls, hypothetically reflecting that the theory of the neural 'model' may give possibly explanation for MTD and particularly for imbalanced laryngeal muscle activation in MTD. In MTD, abnormal sensory feedback (such as poor voice quality) may trigger the neural "model' to stimulate new patterns of muscle activation and alter sensory perception (Figure 4.1B). In particular, abnormal sensory feedback generates an error signal between prediction of the sensory outcome of phonation and incoming sensory feedback. The error signal updates the neural 'model' that in turn generate corrective commands to the motor controller and change sensory perception. Altered descending motor cortical signals stimulate laryngeal motorneurons in the brainstem which might result in excessive tension of (para)laryngeal muscles or recruit muscles that are not ordinarily active. A relationship between the laryngeal motor control impairments and pathophysiology of MTD may be seen. Neural

impulses from the areas that control the execution of laryngeal movements, such as the precentral gyrus, SFG, MFG, midbrain PAG, brainstem and cerebellum, hypothetically may cause muscle tension that can disrupt phonation and produce symptoms of MTD. Simultaneously altered sensory perception might make the brain insensitive to the normal feedback even when irritants are no longer present. Thus, the pathophysiology of MTD may be viewed as a processing of abnormal sensory information throughout intact internal prediction/correction mechanism that results in updating or creating a new neural "model", altering muscle activation patterns and opening sensory channels for abnormal sensory inputs. In our study, lower neural activity in the sensory control-related areas such as STG, MTG, and inferior parietal lobe may reflect suppression in these areas. Neural response suppression in these areas, on the one hand, may occur due to F-high was decreased in patients with MTD according to the acoustic analysis. On the other hand, neural response suppression in these areas might make the brain insensitive to the normal feedback. We also suggest that the neuroplastic changes [106, 107] in the brain areas responsible to phonation control [27] (Figure 4.1B) may support the symptoms of MTD. Furthermore, the updated neural 'model' generates corrective commands to the motor controller (Figure 4.1B) resulting in altered descending motor cortical signals. In our study, higher neural activity in the laryngeal motor control-related areas such as precentral gyrus, SFG, MFG, IFG, midbrain, brainstem, and cerebellum alters descending motor cortical signals and stimulates laryngeal motorneurons in the brainstem that may result in laryngeal tension and voice symptoms in patients with MTD.

The present fMRI study also identified problems with the experimental stimuli and/or procedures. The aim of this study was to investigate brain activity during phonation in women with MTD in comparison with healthy controls in three conditions: comfortable pitch, high pitch, and prolonged exhalation. However, measurements of the vocal quality were not implemented in this fMRI study. During the fMRI procedure it was not possible to make audio recordings of phonations. Therefore the actual difference in fundamental frequency between high pitch and comfortable pitch could not be determined. Before the fMRI scanning, experienced speech therapist explained to all participants how to produce a tasks during fMRI study. For future research, we recommend using voice recordings within the fMRI setup. Furthermore, voice recording during stroboscopy is necessary to compare data.

Another limitation of the present study was that a test of reproducibility was not performed prior to the fMRI study. In the previous fMRI studies, a test of reproducibility has been performed under a number of different experimental paradigms and has reported good reproducibility of data. These fMRI paradigms included: visual stimulation, motor task, and cognitive tasks [108, 107]; sensorimotor tasks [110-111]; or learning tasks [112]. In our study, we did not perform a test of reproducibility because of using a simple fMRI paradigm and did not perform multi-site or multi-scanning session scans. Although a test of reproducibility has been performed in the previous fMRI studies, Friedman et al [111] suggested carrying out reproducibility studies prior to the fMRI study involving the main and original scientific hypothesis, especially when performing multi-site or multi-scanning session scans. Doing so may reveal sources of instability that would introduce a significant variance into the data, and also define if certain statistical benchmarks are met relevant to reproducibility and reliability of data. However, this was a limitation in this study, and future work in this area should include a test of reproducibility performed prior to the fMRI study in order to improve the results.

## 5. Conclusion

The neuroimaging data in this study revealed that MTD patients control phonation by use of the auditory, motor, frontal, parietal, and subcortical areas that are similar to those used during phonation control by healthy subjects. However, *higher* neural activity in the laryngeal *motor* control-related areas such as precentral gyrus, SFG, MFG, IFG, midbrain, and cerebellum as well as the *lower* neural activity in the sensory control-related areas such as STG, MTG, inferior parietal lobe may affect the laryngeal sensorimotor control and result in laryngeal tension and voice symptoms in patients with MTD. Even with a small number of participants in the MTD group, we were able to locate brain regions important to phonation control [42-44, 47, 113-115], and to compare our findings with those of earlier studies [42-44, 47,73, 113-115]. We also suggested that the setup conditions of future fMRI experiments should be modified in order to make vocal pitch recording possible or to rely on fixed vocal pitches. Moreover, future work in this area should include a test of reproducibility performed prior to the fMRI study in order to improve the study results. An updated study protocol should provide further insight in the neural mechanisms of phonation related to laryngeal control in patients with MTD. In addition, future studies should relate routine voice

diagnostic behavioral measures (i.e. perceptual, acoustic, and aerodynamic) to brain imaging data to better understand the relationship between current clinical voice measures and the underlying neural events subserving disordered voice. A better understanding of voice production, from central sensorimotor control to the contribution of the peripheral subsystems, will help to establish biomarkers and lead to customised treatment plans, which might lead to improved clinical outcomes in treatment-seeking populations.

## 6. References


**1.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Brain Activity During Phonation in Women With Muscle Tension Dysphonia: An fMRI Study. *Journal of Voice.* 2017.

**2.** Van Houtte E, Van Lierde K, D'haeseleer E, Claeys S. The prevalence of laryngeal pathology in a treatment□seeking population with dysphonia. *The Laryngoscope.* 2010;120:306-312.

**3.** Van Lierde K, Claeys S, De Bodt M, Van Cauwenberge P. Long-term outcome of hyperfunctional voice disorders based on a multiparameter approach. *Journal of Voice.* 2007;21:179-188.

**4.** Boone DR, McFarlane SC, Von Berg SL, Zraick RI. The voice and voice therapy. 2005.

**5.** Stemple JC, Glaze LE, Gerdeman BK. *Clinical voice pathology: Theory and management*: Cengage Learning; 2000.

**6.** Van Houtte E, Van Lierde K, Claeys S. Pathophysiology and treatment of muscle tension dysphonia: a review of the current knowledge. *Journal of Voice.* 2011;25:202-207.

**7.** Roy N. Functional dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2003;11:144-148.

**8.** Dworkin JP, Meleca RJ, Abkarian G. Muscle tension dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2000;8:169-173.

**9.** Van Houtte E, Claeys S, D'haeseleer E, Wuyts F, Van Lierde K. An examination of surface EMG for the assessment of muscle tension dysphonia. *Journal of Voice.* 2013;27:177-186.

**10.** Van Houtte E, Van Lierde K, D'haeseleer E, Van Imschoot B, Claeys S. UES Pressure During Phonation Using High-resolution Manometry and 24-h Dual-probe pH-metry in Patients with Muscle Tension Dysphonia. *Dysphagia.* 2012;27:198-209.

**11.** Lowell SY, Kelley RT, Colton RH, Smith PB, Portnoy JE. Position of the hyoid and larynx in people with muscle tension dysphonia. *The Laryngoscope.* 2012;122:370-377.

**12.** Von Helmholtz H. *Handbuch der physiologischen Optik*: Voss; 1867.

**13.** Ludlow CL. Laryngeal Reflexes: Physiology, Technique, and Clinical Use. *Journal of Clinical Neurophysiology.* 2015;32:284-293.

**14.** Wyke B. Reflexogenic contributions to vocal fold control systems. In: Titze IR, Ronald CS, eds. *Vocal fold physiology: biomechanics, acoustics and phonatory control* The Denver Center for the Performing Arts, Inc., Denver, Colorado: College-Hill; 1983:138-141.

**15.** Ludlow CL. Central Nervous System Control of Voice and Swallowing. *Journal of Clinical Neurophysiology.* 2015;32:294-303.

**16.** Sundberg J. What's so special about singers? *Journal of voice.* 1990;4:107-119.

**17.** Bouhuys A, Mead J, Proctor DF, Stevens KN. Pressure□flow events during singing. *Annals of the New York Academy of Sciences.* 1968;155:165-176.

**18.** Davis PJ, Zhang SP, Winkworth A, Bandler R. Neural control of vocalization: Respiratory and emotional influences. *Journal of Voice.* 1996;10:23-38.

**19.** Peck KK, Galgano JF, Branski RC, et al. Event-related functional MRI investigation of vocal pitch variation. *Neuroimage.* 2009;44:175-181.

**20.** Finnegan EM, Luschei ES, Hoffman HT. Modulations in respiratory and laryngeal activity associated with changes in vocal intensity during speech. *Journal of Speech, Language, and Hearing Research.* 2000;43:934-950.

**21.** Guenther FH. Cortical interactions underlying the production of speech sounds. *Journal of communication disorders.* 2006;39:350-365.

**22.** Jones JA, Munhall K. The role of auditory feedback during phonation: studies of Mandarin tone production. *Journal of Phonetics.* 2002;30:303-320.

**23.** Houde JF, Niziolek C, Kort N, Agnew Z, Nagarajan SS. Simulating a state feedback model of speaking. *10th International Seminar on Speech Production*2014:202-205.

**24.** Desmurget M, Grafton S. Forward modeling allows feedback control for fast reaching movements. *Trends in cognitive sciences.* 2000;4:423-431.

**25.** Wolpert DM, Ghahramani Z, Jordan MI. An internal model for sensorimotor integration. *Science-AAAS-Weekly Paper Edition.* 1995;269:1880-1882.

**26.** Hickok G. Computational neuroanatomy of speech production. *Nature Reviews Neuroscience.* 2012;13:135-145.

**27.** Pynn LK, DeSouza JF. The function of efference copy signals: implications for symptoms of schizophrenia. *Vision research.* 2013;76:124-133.

**28.** Hickok G, Houde J, Rong F. Sensorimotor integration in speech processing: computational basis and neural organization. *Neuron.* 2011;69:407-422.

**29.** Cramer SC, Sur M, Dobkin BH, et al. Harnessing neuroplasticity for clinical applications. *Brain.* 2011;134:1591-1609.

**30.** Ludlow CL, Hoit J, Kent R, et al. Translating principles of neural plasticity into research on speech motor control recovery and rehabilitation. *Journal of Speech, Language, and Hearing Research.* 2008;51:S240-S258.

**31.** Brosh I, Barkai E. Learning-induced long-term synaptic modifications in the olfactory cortex. *Current neurovascular research.* 2004;1:389-395.

**32.** Tinazzi M, Zanette G, Volpato D, et al. Neurophysiological evidence of neuroplasticity at multiple levels of the somatosensory system in patients with carpal tunnel syndrome. *Brain.* 1998;121:1785-1794.

**33.** Urasaki E, Genmoto T, Wada S-i, Yokota A, Akamatsu N. Dynamic changes in area 1 somatosensory cortex during transient sensory deprivation: A preliminary study. *Journal of clinical neurophysiology.* 2002;19:219-231.

**34.** Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *The Journal of neuroscience.* 1998;18:7000-7007.

**35.** Bellemare F, Woods J, Johansson R, Bigland-Ritchie B. Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *Journal of Neurophysiology.* 1983;50:1380-1392.

**36.** Urberg-Carlson KE. *Muscle tension dysphonia as a disorder of motor learning*, University of Minnesota; 2013.

**37.** Özdemir E, Norton A, Schlaug G. Shared and distinct neural correlates of singing and speaking. *Neuroimage.* 2006;33:628-635.

**38.** Loucks TM, Poletto CJ, Simonyan K, Reynolds CL, Ludlow CL. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. *Neuroimage.* 2007;36:131-143.

**39.** Murphy K, Corfield D, Guz A, et al. Cerebral areas associated with motor control of speech in humans. *Journal of Applied Physiology.* 1997;83:1438-1447.

**40.** Brown S, Laird AR, Pfordresher PQ, Thelen SM, Turkeltaub P, Liotti M. The somatotopy of speech: phonation and articulation in the human motor cortex. *Brain and cognition.* 2009;70:31-41.

**41.** Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cerebral Cortex.* 2008;18:837-845.

**42.** Zarate JM, Zatorre RJ. Experience-dependent neural substrates involved in vocal pitch regulation during singing. *Neuroimage.* 2008;40:1871-1887.

**43.** Zarate JM, Wood S, Zatorre RJ. Neural networks involved in voluntary and involuntary vocal pitch regulation in experienced singers. *Neuropsychologia.* 2010;48:607-618.

**44.** Flagmeier SG, Ray KL, Parkinson AL, et al. The neural changes in connectivity of the voice network during voice pitch perturbation. *Brain and language.* 2014;132:7-13.

**45.** Parkinson AL, Behroozmand R, Ibrahim N, Korzyukov O, Larson CR, Robin DA. Effective connectivity associated with auditory error detection in musicians with absolute pitch. *Frontiers in neuroscience.* 2014;8.

**46.** Lattner S, Meyer ME, Friederici AD. Voice perception: sex, pitch, and the right hemisphere. *Human brain mapping.* 2005;24:11-20.

**47.** Parkinson AL, Flagmeier SG, Manes JL, Larson CR, Rogers B, Robin DA. Understanding the neural mechanisms involved in sensory control of voice production. *Neuroimage.* 2012;61:314-322.

**48.** Simonyan K, Ludlow CL. Abnormal activation of the primary somatosensory cortex in spasmodic dysphonia: an fMRI study. *Cerebral Cortex.* 2010:bhp023.

**49.** Haslinger B, Erhard P, Dresel C, Castrop F, Roettinger M, Ceballos-Baumann A. "Silent event-related" fMRI reveals reduced sensorimotor activation in laryngeal dystonia. *Neurology.* 2005;65:1562-1569.

**50.** Simonyan K, Tovar-Moll F, Ostuni J, et al. Focal white matter changes in spasmodic dysphonia: a combined diffusion tensor imaging and neuropathological study. *Brain.* 2008;131:447-459.

**51.** Ali SO, Thomassen M, Schulz GM, et al. Alterations in CNS activity induced by botulinum toxin treatment in spasmodic dysphonia: an H215O PET study. *Journal of Speech, Language, and Hearing Research.* 2006;49:1127-1146.

**52.** Kiyuna A, Maeda H, Higa A, Shingaki K, Uehara T, Suzuki M. Brain activity related to phonation in young patients with adductor spasmodic dysphonia. *Auris Nasus Larynx.* 2014;41:278-284.

**53.** Rektorova I, Mikl M, Barrett J, Marecek R, Rektor I, Paus T. Functional neuroanatomy of vocalization in patients with Parkinson's disease. *Journal of the neurological sciences.* 2012;313:7-12.

**54.** Liotti M, Ramig L, Vogel D, et al. Hypophonia in Parkinson's disease neural correlates of voice treatment revealed by PET. *Neurology.* 2003;60:432-440.

**55.** Narayana S, Fox PT, Zhang W, et al. Neural correlates of efficacy of voice therapy in Parkinson's disease identified by performance–correlation analysis. *Human brain mapping.* 2010;31:222-236.

**56.** Galgano JF, Peck KK, Branski RC, et al. Correlation between functional MRI and voice improvement following type I thyroplasty in unilateral vocal fold paralysis—a case study. *Journal of Voice.* 2009;23:639-645.

**57.** Joshi A, Jiang Y, Stemple JC, Archer SM, Andreatta RD. Induced unilateral vocal fold paralysis and recovery rapidly modulate brain areas related to phonatory behavior: a case study. *Journal of Voice.* 2011;25:e53-e59.

**58.** Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage.* 2006;31:1453-1474.

**59.** Pedersen JR, Johannsen P, Bak CK, Kofoed B, Saermark K, Gjedde A. Origin of human motor readiness field linked to left middle frontal gyrus by MEG and PET. *Neuroimage.* 1998;8:214-220.

**60.** Hirano S, Kojima H, Naito Y, et al. Cortical dysfunction of the supplementary motor area in a spasmodic dysphonia patient. *American journal of otolaryngology.* 2001;22:219-222.

**61.** Jürgens U. Neural pathways underlying vocal control. *Neuroscience & Biobehavioral Reviews.* 2002;26:235-258.

**62.** Jürgens U. A study of the central control of vocalization using the squirrel monkey. *Medical engineering & physics.* 2002;24:473-477.

**63.** Simonyan K, Horwitz B. Laryngeal motor cortex and control of speech in humans. *The Neuroscientist.* 2011;17 (2):197–208.

**64.** Laukka P, Åhs F, Furmark T, Fredrikson M. Neurofunctional correlates of expressed vocal affect in social phobia. *Cognitive, Affective, & Behavioral Neuroscience.* 2011;11:413-425.

**65.** Jürgens U. The neural control of vocalization in mammals: a review. *Journal of Voice.* 2009;23:1-10.

**66.** Ito M. Mechanisms of motor learning in the cerebellum. *Brain research.* 2000;886:237-245.

**67.** Sachin S, Kumaran SS, Singh S, et al. Functional mapping in PD and PSP for sustained phonation and phoneme tasks. *Journal of the neurological sciences.* 2008;273:51-56.

**68.** Schulz G, Varga M, Jeffires K, Ludlow C, Braun A. Functional neuroanatomy of human vocalization: an H215O PET study. *Cerebral cortex.* 2005;15:1835-1847.

**69.** Belin P, Zatorre RJ, Lafaille P, Ahad P, Pike B. Voice-selective areas in human auditory cortex. *Nature.* 2000;403:309-312.

**70.** Bamiou D-E, Musiek FE, Luxon LM. The insula (Island of Reil) and its role in auditory processing: literature review. *Brain research reviews.* 2003;42:143-154.

**71.** Riecker A, Ackermann H, Wildgruber D, Dogil G, Grodd W. Opposite hemispheric lateralization effects during speaking and singing at motor cortex, insula and cerebellum. *Neuroreport.* 2000;11:1997-2000.

**72.** Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of cognitive neuroscience.* 2000;12:1-47.

**73.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2017;31:118. e121-118. e128.

**74.** Miyapuram KP. Introduction to fMRI: experimental design and data analysis. 2008.

**75.** Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg M, Turner R. Event-related fMRI: characterizing differential responses. *Neuroimage.* 1998;7:30-40.

**76.** Rosen CA, Lee AS, Osborne J, Zullo T, Murry T. Development and validation of the Voice Handicap Index□10. *The Laryngoscope.* 2004;114:1549-1556.

**77.** Remacle M. The contribution of videostroboscopy in daily ENT practice. *Acta oto-rhino-laryngologica Belgica.* 1995;50:265-281.

**78.** Wuyts FL, De Bodt MS, Molenberghs G, et al. The Dysphonia Severity IndexAn Objective Measure of Vocal Quality Based on a Multiparameter Approach. *Journal of Speech, Language, and Hearing Research.* 2000;43:796-809.

**79.** Hirano M. Psycho-acoustic evaluation of voice: GRBAS scale. Clinical Examination of voice. Wien: Springer Verlag; 1981.

**80.** Hirano M. *Clinical examination of voice*: Springer; 1981.

**81.** Timmermans B, De Bodt MS, Wuyts FL, Van de Heyning PH. Analysis and evaluation of a voice-training program in future professional voice users. *Journal of Voice.* 2005;19:202-210.

**82.** Raes J, Wuyts FL, de Bodt M, Clement P. The Dysphonia Severity Index used with a percentage scale. *Stem-, spraak-en taalpathologie.* 2005;11.

**83.** Poburka BJ. A new stroboscopy rating form. *Journal of Voice.* 1999;13:403-413.

**84.** Roy N, Bless DM, Heisey D. Personality and voice disorders: a multitrait-multidisorder analysis. *Journal of Voice.* 2000;14:521-548.

**85.** Roy N, Bless DM. Personality Traits and Psychological Factors in Voice PathologyA Foundation for Future Research. *Journal of Speech, Language, and Hearing Research.* 2000;43:737-748.

**86.** Morrison M, Nichol H, Rammage L. Diagnostic criteria in functional dysphonia. *The Laryngoscope.* 1986;96:1-8.

**87.** Morrison M, Rammage L, Belisle G, Pullan C, Nichol H. Muscular tension dysphonia. *The Journal of otolaryngology.* 1983;12:302-306.

**88.** Rubin JS, Lieberman J, Harris TM. Laryngeal manipulation. *Otolaryngologic Clinics of North America.* 2000;33:1017-1034.

**89.** Koufman JA, Blalock PD. Classification and approach to patients with functional voice disorders. *Annals of Otology, Rhinology & Laryngology.* 1982;91:372-377.

**90.** Angsuwarangsee T, Morrison M. Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice.* 2002;16:333-343.

**91.** Altman KW, Atkinson C, Lazarus C. Current and emerging concepts in muscle tension dysphonia: a 30-month review. *Journal of Voice.* 2005;19:261-267.

**92.** Rubin JS, Sataloff RT, Korovin GS. *Diagnosis and treatment of voice disorders.* 4th ed. San Diego, CA: Plural Publishing, Inc; 2014.

**93.** Koufman J, Blalock P. Functional voice disorders. *Otolaryngologic Clinics of North America.* 1991;24:1059-1073.

**94.** Amaro E, Barker GJ. Study design in fMRI: basic principles. *Brain and cognition.* 2006;60:220-232.

**95.** Talairach J, Tournoux P. *Co-planar Stereotaxic Atlas of the Human Brain: 3-dimensional Proportional System : an Approach to Cerebral Imaging.* 1st ed. Stuggart, Germany: Thieme; 1988.

**96.** Calhoun VD, Liu J, Adalı T. A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. *Neuroimage.* 2009;45:S163-S172.

**97.** Bullmore E, Brammer M, Williams SC, et al. Statistical methods of estimation and inference for functional MR image analysis. *Magnetic Resonance in Medicine.* 1996;35:261-277.

**98.** Lieberman MD, Cunningham WA. Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social cognitive and affective neuroscience.* 2009:nsp052.

**99.** Hickok G, Buchsbaum BR, Humphries C, Muftuler T. Auditory–motor interaction revealed by fMRI: speech, music, and working memory in area Spt. *Cognitive Neuroscience, Journal of.* 2003;15:673-682.

**100.** Picard N, Strick PL. Motor areas of the medial wall: a review of their location and functional activation. *Cerebral cortex.* 1996;6:342-353.

**101.** Jürgens U, von Cramon D. On the role of the anterior cingulate cortex in phonation: A case report. *Brain and language.* 1982;15:234-248.

**102.** Goldberg II, Harel M, Malach R. When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron.* 2006;50:329-339.

**103.** Sundberg J. The science of the singing voiceNorthern Illinois University Press. *Dekalb, IL.* 1987.

**104.** Sundberg J. Formant technique in a professional female singer. *Acta Acustica united with Acustica.* 1975;32:89-96.

**105.** Porter BA, Khodaparast N, Fayyaz T, et al. Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. *Cerebral Cortex.* 2011;22:2365-2374.

**106.** Malandraki GA, Johnson S, Robbins J. Functional MRI of swallowing: from neurophysiology to neuroplasticity. *Head & neck.* 2011;33:S14-S20.

**107.** Kleim JA, Jones TA. Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *Journal of speech, language, and hearing research.* 2008;51:S225-S239.

**108.** Casey B, Cohen JD, O'Craven K, et al. Reproducibility of fMRI results across four institutions using a spatial working memory task. *Neuroimage.* 1998;8:249-261.

**109.** Manoach DS, Halpern EF, Kramer TS, et al. Test-retest reliability of a functional MRI working memory paradigm in normal and schizophrenic subjects. *American Journal of Psychiatry.* 2001;158:955-958.

**110.** Loubinoux I, Carel C, Alary F, et al. Within-session and between-session reproducibility of cerebral sensorimotor activation: a test–retest effect evidenced with functional magnetic resonance imaging. *Journal of Cerebral Blood Flow & Metabolism.* 2001;21:592-607.

**111.** Friedman L, Stern H, Brown GG, et al. Test–retest and between□site reliability in a multicenter fMRI study. *Human brain mapping.* 2008;29:958-972.

**112.** Aron AR, Gluck MA, Poldrack RA. Long-term test–retest reliability of functional MRI in a classification learning task. *Neuroimage.* 2006;29:1000-1006.

**113.** Toyomura A, Koyama S, Miyamaoto T, et al. Neural correlates of auditory feedback control in human. *Neuroscience.* 2007;146:499-503.

**114.** Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. *Neuroimage.* 2008;39:1429-1443.

**115.** Parkinson AL, Korzyukov O, Larson CR, Litvak V, Robin DA. Modulation of effective connectivity during vocalization with perturbed auditory feedback. *Neuropsychologia.* 2013;51:1471-1480.

**116.** Shaw GY, Sechtem P, Searl J, Dowdy ES. Predictors of laryngeal complications in patients implanted with the Cyberonics vagal nerve stimulator. *Annals of Otology, Rhinology & Laryngology.* 2006;115:260-267.

# CHAPTER 5

# BRAIN ACTIVITY DURING PHONATION IN HEALTHY FEMALE SINGERS WITH SUPRAGLOTTIC COMPRESSION: AN FMRI PILOT STUDY

This chapter will present the readers with the results for fMRI pilot study of brain activity during phonation in healthy women with singing experience using the proposed fMRI protocol described in Chapter 3.

**Abstract**

This pilot study evaluated the usability of functional magnetic resonance imaging (fMRI) to detect brain activation during phonation in healthy female singers with supraglottic compression. Four healthy female classical singers (mean age: 26 years) participated in the study. All subjects had normal vocal folds and vocal characteristics and showed supraglottic compression. The fMRI experiment was carried out using a block design paradigm. Brain activation during phonation and exhalation was analyzed using Brain Voyager software. An fMRI data analysis showed a significant effect of phonation control in the bilateral pre/postcentral gyrus, and in the frontal, cingulate, superior and middle temporal gyrus, as well as in the parietal lobe, insula, lingual gyrus, cerebellum, thalamus, and brainstem. These activation areas are consistent with previous reports using other fMRI protocols. In addition, a significant effect of phonation compared to exhalation control was found in the bilateral superior temporal gyrus, and the pre/postcentral gyrus. This fMRI pilot study allowed to detect a normal pattern of brain activity during phonation in healthy female singers with supraglottic compression using the proposed protocol. However, the pilot study detected problems with the experimental material/procedures that would necessitate refining the fMRI protocol. The phonation tasks were not capable to show brain activation difference between high-pitched and comfortable phonation. During the fMRI procedure there was no possibility to make audio recordings of phonations. Therefore the actual difference between high pitch and comfortable pitch could not be determined..

## 1. Introduction

Human phonation is a laryngeal motor behavior that extends from reflexive and unlearned limbic laryngeal actions [1-3] to controlled and coordinated, highly skilled vocal movements to support speech and/or singing [4, 5]. Phonation requires coordination of the respiratory, laryngeal, and articulatory systems. A component of normal phonation is the variation of voice pitch (habitual, high, and low) which requires a complex interplay between respiratory (i.e., subglottic pressure) and laryngeal control [6]. Pitch variation depends on the interactions between intrinsic and extrinsic laryngeal muscles [7-9] which influence the properties of the vocal folds (i.e., the sound source system) and in this way control the F0, as well as the interplay between F0 and vocal tract resonance frequencies (i.e., F0 adjustment). Activity of the intrinsic cricothyroid and thyroarytenoid muscles [6, 10, 11] and the extrinsic sternohyoid [12] muscle increases as F0 increases during vocalization. High-pitched phonation is associated with increased tracheal pressure [13] as well as increments of loudness and glottal airflow [14]. Sensorimotor integration control plays an important role in the feedback-based adjustments during phonation [15]. Moreover, vocal training also may result in changes in laryngeal activity and its control [16].

People with vocal problems (e.g. functional dysphonia) often have limited pitch ranges due to laryngeal compression and postural problems during phonation [17-20]. Medio-lateral (M-L) and anterior-posterior (A-P) laryngeal compression is an endoscopic sign of abuse or misuse of the vocal mechanism and/or vocal hyperfunction [21-26] and is commonly reported in patients with functional dysphonia such as muscle tension dysphonia (MTD) [27]. MTD is a common type of functional dysphonia that is clinically characterized by increased laryngeal muscle tension [23-25, 28, 29] and is often associated with vocal pitch range limitation despite the absence of *primary* organic or neurologic laryngeal disorders. MTD is commonly seen in vocally untrained people [28] and tends to develop among middle-aged women who use their voice as a professional tool [30]. Moreover, recent studies have demonstrated that laryngeal compression may be a regular laryngeal behavior during normal speaking and singing [26, 27, 31-37]. Up to now, no studies were able to identify neural biomarkers that determine supraglottic compression and help to understand whether laryngeal compression is either a dysfunctional or a normal laryngeal behavior.

Neuroimaging techniques have become important tools to describe neural networks associated with laryngeal control of phonation for voice, speech, and/or singing [38-42]. These studies identified the sensorimotor cortex region (corresponding to Brodmann's area (BA) 1, 2, 3, or 4), premotor cortex region (BA 6, 8), superior temporal gyrus (STG) (BA 22,41, 42), insula (BA 13), cingulate gyrus/cortex, supramarginal gyrus (BA 40), lingual gyrus (BA 18, 19), thalamus, cerebellum, midbrain, and basal ganglia as key regions in the functional network of non-disordered phonation [38-41, 43, 44]. More specifically, as defined by functional brain imaging, the sensorimotor cortex region functionally includes the primary motor cortex (BA 4) and S1 (BA 1, 2, and 3), and is anatomically located on/in the pre/postcentral gyrus and central sulcus [45]. In addition, the premotor cortex region functionally includes the premotor cortex as well as a supplementary motor area and is anatomically located on/in the precentral gyrus and superior/middle/inferior frontal gyrus (SFG, MFG, IFG) [45]. Recent functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies identified the primary motor cortex, supplementary motor area, cingulate cortex, and inferior parietal lobe as key regions in the functional network to sing single notes [46], melodies [47], or an Italian aria [48]. Moreover, neuroimaging studies were able to show that singers and non-singers recruited similar brain areas in simple singing, i.e. bilateral auditory cortices, cingulate cortex, supplementary motor area, primary motor cortices, premotor cortex, insula, primary somatosensory cortex (S1), thalamus, and cerebellum [49]. However, none of the previous studies specifically investigated phonation control in healthy singers with supraglottic compression to determine the neural mechanism behind laryngeal function in this population during regular laryngeal behavior. On one hand, several studies evaluated supraglottic activity in professional voice users (healthy classical singers, rock singers, and theater actors) using routine diagnostic measures (i.e. perceptual, acoustic, aerodynamic, and flexible endoscopic laryngeal evaluation) [31, 32, 35-37, 50-55]. A study by Mayerhoff et al [31] revealed supraglottic activity (medial and/or A-P compression) in healthy singers during loud phonation of the vowel /a/. Male subjects demonstrated a higher degree of A-P compression. Moreover, A-P and medial compression are significantly correlated and occur simultaneously in these subjects [31]. Guzman et al [50] have shown vocal hyperfunction manifested by laryngeal postural changes including high vertical positions, pharyngeal compression, and laryngeal supraglottic compression during singing and speaking in healthy rock singers. Another study found a certain degree of A-P laryngeal compression, medial compression,

pharyngeal constriction, and high vertical laryngeal position in healthy pop singers while singing in several styles (pop, rock, and jazz). Moreover, rock singing showed the highest degree of both laryngeal and pharyngeal activities [55]. On the other hand, there are no studies that evaluate the neurophysiological mechanism of laryngeal compression during phonation in healthy singers without voice disorders and with supraglottic compression. This has been the rationale to investigate the neural control of phonation in healthy singers with supraglottic compression to identify neural biomarkers that may determine supraglottic compression and help to understand whether laryngeal compression is either a dysfunctional or a normal laryngeal behavior.

The aim of this study was to determine if the proposed fMRI protocol is sensitive enough to define a widespread network of activation associated with phonation in healthy female singers with normal vocal characteristics and supraglottic compression. A pilot study was done to fine tune the experimental tasks and to identify any problems with the experimental stimuli or procedures.

In the pilot study, we implemented a paradigm consisting of sustained phonation of the sound /i/ on different pitch levels and prolonged exhalation tasks [42]. The phonation tasks were meant to explore the interplay between respiratory and laryngeal control, whereas the exhalation tasks explored respiratory control separately. Additionally, the phonation tasks revealed the neural control associated with changes in respiratory and laryngeal adjustments to obtain vocal pitch changes (comfortable and high). Comfortable phonation (i.e., habtual $F_0$) relies on a balance of muscle tension in both the voicing and respiratory system. High pitch phonation produced at high intensity relies on maximal/highest muscular activity of the intrinsic and extrinsic laryngeal muscles and the respiratory system. Hence, we expected differences in brain activity pertaining to pitch variation (comfortable and high) control during phonation. Laryngeal control maps were generated by subtraction of the exhalation condition from the phonation condition. This approach is based on a study by Loucks et al [40] which showed that the neural control of exhalation for phonation is similar to the neural control of voluntary exhalation in healthy people, except for a difference in STG activation due to the auditory feedback. These results were obtained during fMRI data analysis by subtracting patterns of neural control for voluntary exhalation from those during for phonation, considering the fact that if activity in a particular region of the brain during one task is greater than during another task, this particular region

of the brain is involved in specific task-related activity [56, 57]. We hypothesized that the proposed fMRI protocol could detect brain activation during phonation in healthy female singers with supraglottic compression.

## 2. Material and methods

### Ethics Approval

The Ethics Committee of Ghent University Hospital approved (B670201420193) the study protocol.

### Participants

Four healthy female classical singers participated in the study. Inclusion criteria were (1) no history of vocal pathology in the past year, (2) at least 5 years of training in classical singing, (3) soprano voice type, (4) endoscopic sign of laryngeal compression, (5) age between 21-45 years, (6) female gender, (7) right-handedness, (8) being a native speaker of Dutch, (9) no history of neurological or psychiatric disease. Four singers (mean age: 26 years, age range: 22-33) met the inclusion criteria and were recruited to participate in the study. The average length of voice training of the subjects was 8,25 years, with a range of 5-12 years. The rationale to include only adult healthy women with a soprano voice was to reduce intra-group variance during fMRI data analysis. Participants were recruited from the employees of Ghent University using an open ad. Written informed consent was obtained from all participants. All were asked to undergo flexible nasolaryngoscopy to confirm the absence of laryngeal pathology and the presence of laryngeal supraglottic compression.

### Questionnaires and voice handicap index

Before scanning, all participants filled in a Pre-scan MRI-safety questionnaire, the Edinburgh Handedness Inventory measurement scale, and a Personal History Questionnaire. These questionnaires were used to select participants who satisfy inclusion criteria, such as fMRI compatibility, medical history,

and lifestyle, and other participant characteristics. The psychosocial impact of vocal quality, as perceived by the subject, was measured by means of the validated Dutch translation of the Voice Handicap Index-10 (VHI) [58]. This instrument assesses a subject's perception of disability, handicap, and distress resulting from voice difficulties. It consists of 10 questions that cover emotional (2 questions), physical (3 questions), and functional (5 questions) aspects of the respondent's voice. The questions are rated on a 5-point ordinal scale: never (0), almost never (1), sometimes (2), almost always (3), and always (4). The total score ranges from 0 (no problem perceived) to 40. After scanning, participants filled in a Post-Scan MRI-Checklist which asked for information on effects of the MRI equipment and its environment (i.e., magnetic field, acoustic noise).

## Clinical examination and voice assessment protocol

The same otorhinolaryngologist (S.C.) and speech therapist (E.D.) examined each subject clinically following a standard evaluation protocol. This protocol included the ENT (ear, nose, and throat) evaluation and videonasolaryngoscopic examination [59]. Clinical examination included focal palpation of tension around the larynx. The voice assessment protocol included a perceptual rating of the voice during connected speech by using the GRBASI scale and an objective vocal quality evaluation by means of the dysphonia severity index (DSI) [60]. The GRBASI scale consists of five well-defined parameters: G (overall grade of hoarseness), R (roughness), B (breathiness), A (asthenic), and S (strained) [61, 62]. A sixth parameter I for instability of the voice was added later to the Likert scale [63]. A four-point rating scale (0: normal, 1: slight, 2: moderate, and 3: severe) is used to indicate the grade of every parameter (table 1). The objective parameters of the voice assessment protocol included the frequency range (F0 low-F0 high), the intensity range (I low-I high), aerodynamics (maximum phonation time, MPT, and vital capacity, VC), and the acoustic microperturbations (Jitter and Shimmer) of voice during phonation of the vowel sound /a/ and /i/. The voice range was measured using the voice range profile module from the Computerized Speech Lab Model 4500 (CSL$^{TM}$, KayPENTAX, Lincoln Park, NJ). Recordings were made using a hand-held unidirectional condenser microphone (Samson, C01U, mouth-to-microphone distance = 7 cm). The acoustic analysis was performed with the Multi Dimensional Voice Program (MDVP) from the CSL. All measurements took place in a sound-treated room. Based on these results, the DSI was calculated: (0.13 ×

MPT) + (0.0053F0 high) − (0.26 × Ilow) − (1.18 × Jitter) + 12.4 [60]. DSI outcomes range from +5 (for perceptually normal voices) to -5 (for dysphonic voices) and the more negative DSI outcomes are, the worse vocal quality end results become [60]. All participants had a DSI value corresponding to a normal voice quality (mean DSI of the vowel /a/: +4.6, DSI range: 2,9-5,9; mean DSI of the vowel /i/: +5.3, DSI range: 2,3-7) [60]. In addition, voice samples based on the production of sustained vowels /a/ and /i/ were used to determine the habitual fundamental frequency (F0) and the highest frequency (F0-highest) for each subject (mean F0: 245,2 and mean F0-highest: 973,2 of the vowel /i/ and mean F0: 228,5 and mean F0-highest: 756,9 of the vowel /a/) (table 5.1).

| **Vocal assessment protocol** | **Mean (Standard deviation (SD)) sustained vowel /a/** | **Mean (SD) sustained vowel /i/** |
|---|---|---|
| **Vocal range** | | |
| Lowest intensity (dB) | 56.3 (2,2) | 54,1 (3) |
| Highest intensity (dB) | 103,8 (3) | 94,3 (3,7) |
| Lowest frequency (Hz) | 145 (14,9) | 124,9 (45,6) |
| Highest frequency (Hz) | 743,9 (420,6) | 799,3 (137) |
| Fundamental frequency F0 (Hz) | 223,6 (8,5) | 211 (16) |
| **Aerodynamics** | | |
| Maximum phonation time (sec) | 18,4 (5,2) | 22,6 (5,1) |
| Vital capacity ($cm^3$) | 2387,5 (432,8) | 2610 (520) |
| **Acoustic analysis** | | |
| Jitter (%) | 1,6 (0,9) | 1,6 (1) |
| Shimmer (%) | 3,6 (0,5) | 2,7 (0,9) |
| **DSI** | 4,2 (0,5) | 3,8 (2,0) |
| **VHI-10 (**VHI score per question ranging from 0 to 4) | **Mean** | **SD** |
| VHI functional | 1,3 | 1,3 |
| VHI physical | 3,8 | 1,3 |
| VHI emotional | 0 | 0 |
| VHI total (0-40) | 2,8 | 3,8 |
| GRBASI | $G_0R_0B_0A_0S_0I_0$ | 0 |

**Table 5.1: Voice Assessment Protocol, Voice Handicap Index (VHI) in healthy female singers with supraglottic hyperfunction (n=4).**

Subject selection was also based upon videostrobonasolaryngoscopy. The videostrobolaryngoscopy examination protocol included phonation of the vowel sounds /a/, /i/, and /u/ at

modal/comfortable, low-pitched (soft and loud to assess maximum pliability), and high-pitched voice quality. The following videostroboscopy indicators at modal, low, and high pitch were evaluated: symmetry (symmetrical or asymmetrical), regularity (regular, irregular, or inconsistent), glottal closure (complete, incomplete, or inconsistent), type of gap (longitudinal, posterior, anterior, irregular, oval, or hour-glass), amplitude (increased, normal, reduced, or none), and mucosal wave (normal, reduced, or none) [59]. The female singers have a higher incidence of a small (less than 25% of the length of the vocal folds) posterior chink[64]. A posterior glottal gap between the vocal folds was expected in nasolaryngostroboscopy in this type of population (table 2). Supraglottic activity refers to the positioning and location of structures immediately above the vocal folds. Laryngeal supraglottic compression during videostrobonasolaryngoscopy was established when A-P and/or M-L compression was present. Typically, the visibility of vocal folds was partially affected by the presence of supraglottic activity, since A-P compression is a result of the approximation of arytenoid cartilages to the petiole of the epiglottis and M-L compression happens when there is adduction of vestibular folds. By using the SERF-protocol [65], supraglottic activity was quantified. The SERF form features a laryngeal image with concentric circles superimposed. M-L and A-P constriction were evaluated separately by determining which numbered circle corresponds best to the observed degree of constriction (from 0: no constriction to 4: very severe constriction). Videostrobonasolaryngoscopy evaluations of the participants showed normal laryngeal structure and function during phonation of /u/ at modal/comfortable, low-pitched and high-pitched voice quality. However, during phonation of /i/ and /a/ at modal/comfortable, low-pitched, and high-pitched voice quality, participants had A-P and/or M-L supraglottic compression (Table 5.2).

## Functional magnetic resonance imaging experimental protocol

The fMRI experiment was performed in a block design [42]. All participants were tested under three different conditions: I. COMFORTABLE PHONATION: modal/comfortable prolonged phonation of the vowel /i/ without spreading the lips; II. HIGH-PITCHED PHONATION: high-pitched prolonged phonation of the vowel /i/ without spreading the lips; III. PROLONGED EXHALATION: voluntary sustained 'unvoiced' oral exhalation. Participants were instructed to phonate the vowel /i/ with a habitual pitch and loudness for the comfortable phonation task and with highest pitch and maximal loudness for the

high-pitched phonation task. During comfortable phonation there is a sustained balance of tension in the muscles of phonation and the respiratory system. High phonation is based on a maximal activity of the intrinsic and extrinsic laryngeal muscles and the respiratory musculature. The conditions I to III were performed in randomized order for each participant. The time interval during which the volunteers had to perform a task was visually indicated during 10 seconds by a grey loading bar, whereas resting periods were indicated by a black cross. Before the actual tasks, written instructions indicating the type of task were presented (2 seconds) in the subject's native language (i.e. in Dutch: 'Gewone Stem', 'Hoge Stem' or 'Verlengde Uitademing') as well as a visual cue to start inspiration (2.5 seconds) (i.e. in Dutch: 'Inademen'). All visual commands were generated using a commercially available experiment generator (Presentation, Neurobehavioral Systems Inc., Albany CA, USA) and were reflected in a mirror on the head coil. Subjects were positioned head-first supine inside the magnet bore and fitted with an OptoACTIVE noise cancelling MRI headphone and a FOMRI-III noise cancelling microphone (OptoActive™, Optoacoustics Ltd, Moshav Mazor, Israel). The OptoACTIVE system provided the highest levels of noise reduction and self-monitoring during phonation. However, the OptoACTIVE system did not provide phonatory task recordings free of fMRI-related acoustic noise during scanning. The participant's head was immobilized in the standard head coil using neck cushions to minimize motion artifacts. The subjects were instructed to keep their jaw, lips and tongue motionless while performing the tasks and to keep their jaw slightly open in order to minimize movements during phonation (e.g. movements of orofacial muscles), which might also cause artifacts during fMRI scanning. In addition, participants were asked to reduce articulatory gestures during sustained phonation tasks. The project leader (S.C.) and MRI operator (M.K.) monitored performances throughout the experiment through a control room speaker. In addition, prior to scanning, speech pathologist explained to all participants how to produce a sustained vowel /i/ during 10 seconds using a comfortable as well as a high pitch and to sustain exhalation for the same duration for the fMRI study.

| Videostroboscopic Feature | Phonation of the vowel sounds at three different pitches (F0-low, F0, and F0-high) | | | | | |
|---|---|---|---|---|---|---|
| | /i/ | | /u/ | | /a/ | |
| | n | % | n | % | n | % |
| **Symmetry** | | | | | | |
| Symmetrical | 3 | 75 | 3 | 75 | 3 | 75 |
| Asymmetrical | 1 | 25 | 1 | 25 | 1 | 25 |
| **Regularity** | | | | | | |
| Regular | 4 | 100 | 4 | 100 | 4 | 100 |
| Irregular | - | - | - | - | - | - |
| Inconsistent | - | - | - | - | - | - |
| **Glottic closure** | | | | | | |
| Complete | 1 | 25 | 1 | 25 | 1 | 50 |
| Incomplete | 3 | 75 | 3 | 75 | 2 | 50 |
| **Type glottal gap** | | | | | | |
| Longitudinal | - | - | - | - | - | - |
| Posterior | 3 | 75 | 3 | 75 | 2 | 50 |
| Anterior | - | - | - | - | - | - |
| Oval | - | - | - | - | - | - |
| Hour-glass | - | - | - | - | - | - |
| **Amplitude** | | | | | | |
| Normal | 4 | 100 | 4 | 100 | 4 | 100 |
| Reduced | - | - | - | - | - | - |
| Increased | - | - | - | - | - | - |
| **Mucosal wave** | | | | | | |
| Normal | 4 | 100 | 4 | 100 | 4 | 100 |
| Reduced | - | - | - | - | - | - |
| None | - | - | - | - | - | - |
| **A-P constriction** | | | | | | |
| 0 | - | - | - | - | - | - |
| 1 | 1 | 25 | - | - | 3 | 75 |
| 2 | 3 | 75 | - | - | 1 | 25 |
| 3 | - | - | 3 | 75 | - | - |
| 4 | - | - | 1 | 25 | - | - |
| **M-L constriction** | | | | | | |
| 0 | 3 | 75 | 3 | 75 | 4 | - |
| 1 | 1 | 25 | 1 | 25 | - | - |
| 2 | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - |
| * one participant had slightly asynchronous and irregular vocal folds movements | | | | | | |

**Table 5.2: Videostroboscopic features in healthy female singers with supraglottic hyperfunction (n=4).** Abbreviations: A-P, anterior-posterior constriction (0 no constriction, 4 severe constriction); M-L, medio-lateral constriction (0 no constriction, 4 severe constriction).

## Magnetic resonance imaging acquisition

FMRI images were acquired on a 3 Tesla MR scanner (Siemens Magnetom Trio, Erlangen, Germany) using the standard 32-channel head coil. Initially, an anatomical T1-weighted MR dataset covering the whole head at 1 $mm^3$ isotropic resolution was acquired (high-quality three-dimensional magnetization-prepared rapid acquisition with gradient echo (3D MPRAGE) images, repetition time = 1950 ms, inversion time =1100 ms, echo time = 3.93 ms, flip angle = 12°). An axial T2*-sensitive gradient-echo echo-planar imaging technique with an in-plane resolution of $2x2mm^2$ was used to generate the functional images (repetition time = 2000 ms, echo time = 36 ms, flip angle = 70°, acquisition matrix = 96x128). Forty consecutive sections of 3 mm thickness with a 0.5 mm gap between slices in an axial-to-coronal orientation were acquired. A total of 176 volumes were recorded for experimental run, resulting in a total investigation time of 25 minutes.

## Image analysis steps

All steps of fMRI data preprocessing and fMRI group data analysis were performed using the BrainVoyager QX Version 2.4 software package (Brain Innovation, Maastricht, The Netherlands) [66]. Preprocessing included 3D motion correction, slice timing correction, normalization to a standard echo planar imaging (EPI) template based on the neuroanatomical atlas of Talairach and Tournoux [67], and spatial smoothing on volume time course (VTC) files with a Gaussian kernel for the full width at the half maximum (FWHM) of 8 mm (the voxel size of resultant VTC was $3 \times 3 \times 3$ $mm^3$). A statistical parametric map was calculated using the the general linear model (GLM) approach. For each experiment, a BrainVoyager protocol file (PRT) was derived, representing the onset and duration of the events for the different conditions with the rest period as a baseline. From the created protocols, the design matrices for the calculation of the GLM were defined automatically. In order to account for hemodynamic response, each of the predictors was derived by convolution of the block design with a model for the two gamma hemodynamic response functions [57]. Previously, the GLM design matrix was improved by defining proper noise predictors using the Independent Component Analysis (ICA) approach [68]. After fitting the GLM [69], group t-maps were generated by invoking the RFX-ANCOVA (ANCOVA Random Effect Analysis) tool

and using a subtraction approach [40, 56, 57] for fMRI data analysis of the comfortable phonation, high-pitched phonation and prolonged exhalation as well as for the comparisons between conditions of phonation and prolonged exhalation. Activation maps were generated by thresholding the statistical maps using the false discovery rate (FDR) approach (q(FDR)<0.05) [66, 70].

## 3. Results

### Functional magnetic resonance imaging experiment

Table 5.3 and Figure 5.1 summarize the main fMRI results of the study. Group analysis showed a significant main effect of comfortable phonation compared to rest condition in the right pre/postcentral gyrus, left precentral gyrus, bilateral middle frontal gyrus, cingulate gyrus, bilateral lingual gyrus, bilateral STG extends to the inferior parietal lobe, bilateral cerebellum, brainstem and of high-pitched phonation compared to rest condition in the bilateral pre/postcentral gyrus, bilateral superior frontal gyrus, cingulate gyrus, right lingual gyrus, bilateral STG, thalamus, cerebellum, and brainstem (table 5.3). Activities in the bilateral SFG were shown during the high-pitched phonation compared to the rest period. However, the fMRI data analysis for the high-pitched phonation compared to comfortable phonation did not reveal any significant differences in brain activation between high-pitched phonation and comfortable phonation. Group analyses showed a significant main effect of exhalation compared to rest period in the bilateral precentral gyrus, right lingual gyrus, left supramarginal gyrus, right thalamus, and cerebellum (table 5.3). Comparing phonation (comfortable and high) and prolonged exhalation tasks yielded a significant main effect of phonation in the bilateral pre/postcentral gyrus and STG (Table 5.3, Figure 5.1).

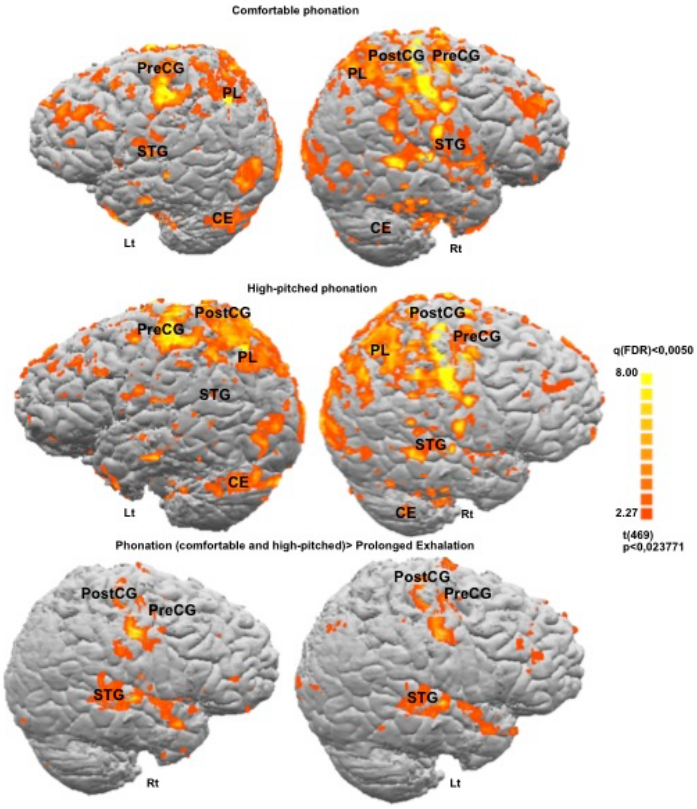


**Figure 5.1: Brain activation during phonation.** Abbreviations: PreCG: precentral gyrus; PosCG: postcentral gyrus; STG: superior temporal gyrus; PL: parietal lobe; CE: cerebellum; Rt: right; Lt: left).

| Brain area (n=4) | BA | Talairach coordinates (x,y,z) | Peak t (469) value | Cluster size ($mm^3$) |
|---|---|---|---|---|
| **COMFORTABLE PHONATION** | | | | |
| Right pre/postcentral gyrus | 3,4,6 | 41;-14;36/52;-11;48 | 15 | 3630 |
| Left precentral gyrus | 4,6 | -41;-16;30 | 8,3 | 1961 |
| Right middle frontal gyrus | 9,10 | 29;15;58 | 4,7 | 143 |
| Left middle frontal gyrus | 9,10 | -26;22;58 | 5,3 | 573 |
| Cingulate gyrus | 24 | 25;-45;25 | 14 | 6848 |
| Right lingual gyrus | 18 | 37;-75;0 | 10 | 2110 |
| Left lingual gyrus | 18 | -37;-75;0 | 10 | 2699 |
| Right superior temporal gyrus (extends to the inferior parietal lobe) | 22, 41, 42 | 57;-27;7 | 10 | 6092 |
| Left superior temporal gyrus (extends to the inferior parietal lobe) | 22, 42 | -57;-27;7 | 13 | 5709 |
| Cerebellum | | 35;-58;-27/-35;-58;-27 | 19 | 13576 |
| Brainstem | | -5;-24;-41 | 9,7 | 1313 |
| **HIGH-PITCHED PHONATION** | | | | |
| Right pre/postcentral gyrus | 4,6 | 46;-8;44 | 14,3 | 3440 |
| Left pre/postcentral gyrus | 3,4-6 | -50;-8;42 | 13 | 5060 |
| Right/Left Middle frontal gyrus | | 26;41;43/-26;41;43 | 13 | 7464 |
| Right/Left Superior frontal gyrus | 8 | 10;38;45/-10;38;45 | 13 | 3212 |
| Cingulate gyrus | 24 | -5;-1;32 | 13 | 186135 |
| Right lingual gyrus | 19 | 37;-75;0 | | |
| Right superior temporal gyrus | 38 | 45;24;-18/28;7;-27 | 17,6/12,3 | 1249/1138 |
| Left superior temporal gyrus | 22,38 | -30;1;-27/-44;20;-22/-62;-14;2 | 14,9/12,04/8,3 | 834/1064/776 |
| Thalamus (ventral posterior lateral nucleus) | | 2;-8;9 | 10 | 3051 |
| Cerebellum | | 2;-44;-29/-2;-44;-29 | 17 | 58983 |
| Brainstem | | 0;-25;-28 | 13 | 9622 |
| **PROLONGED EXHALATION** | | | | |
| Right/Left precentral gyrus | 4,6 | 42;-10;32/46;3;11/-42;-17;37 | 6,5 | 6827 |
| Right lingual gyrus | 19 | 39;-70;-6 | 5,8 | 7477 |
| Left supramarginal gyrus | 40 | -42;-54;49 | 4,7 | 1176 |
| Right thalamus | | 13;-7;6 | 4,9 | 303 |
| Cerebellum | | 11;-35;-14/-19;-34;-16 | 4,8 | 302 |
| **PHONATION (COMFORTABLE, HIGH-PITCHED PHONATION)>EXHALATION** | | | | |
| Right pre/postcentral gyrus | 3,4-6 | 42;-7;30/ 52;-21;48 | 5 | 871 |
| Left pre/postcentral gyrus | 3,4-6 | -42;-7;30-52;-21;48 | 6 | 515 |
| Right superior temporal gyrus | 13,22 | 54;-24;6 | 10,6 | 7787 |
| Left superior temporal gyrus | 13,22 | -53;-42;17 | 9,2 | 1647 |

**Table 5.3: Brain activation during phonation.** Results are presented in Talairach space q(FDR)<0,05.

## 4. Discussion

This pilot study was a first step to detect brain activity during phonation in healthy female singers with normal vocal folds and vocal characteristics and with supraglottic compression using the proposed fMRI protocol. We included four adult healthy female soprano singers without voice disorders and with supraglottic compression. We implemented an experimental paradigm consisting of sustained phonation of /i/ and prolonged exhalation tasks. The phonation tasks in this study explored respiratory and laryngeal control. Additionally, the phonation tasks in this study explored the neural control associated with changes in pitch (comfortable and high). Comfortable phonation is based on maintaining a comfortable balance of activity in the muscles of voicing and the respiratory system. High phonation is based on a maximal activity in the muscles of voicing and respiratory system. We expected brain activity differences related to pitch adaptation. The exhalation tasks explored respiratory control and allowed to generate laryngeal control maps by subtraction of the exhalation condition from the phonation condition [40, 56, 57]. We hypothesized that the proposed fMRI protocol would be suitable to detect brain activation during phonation in healthy female singers with supraglottic compression. We also hypothesized that analysis of the pilot fMRI data might reveal potential problems in experimental material (stimuli and/or procedures) which would necessitate revising the fMRI protocol.

FMRI data analysis showed a significant effect of phonation control in the bilateral pre/postcentral gyrus, frontal gyrus, cingulate gyrus, superior and middle temporal gyrus, parietal lobe, insula, lingual gyrus, cerebellum, thalamus, and brainstem (table 3). These activation areas are consistent with previous reports using other fMRI protocols on phonation control by healthy singers and non-singers [38-44]. In addition, activity in the bilateral SFG was present during the high-pitch phonation task only when compared to the rest period. Goldberg [71] found that when a personal emotional response was required, participants showed activity in the SFG – the brain region associated with self-awareness-related function. In our experiment, activity in the bilateral SFG was present during the high pitch-phonation task, hypothetically reflecting greater emotional activity co-occurring with higher vocal effort required to control

high-pitched phonation. Additionally, to test whether sensory input affects brain activity during vocal pitch modulation, a comparison between comfortable pitch and high pitch phonation was performed. Since pitch modulation is based on modifying laryngeal and respiratory control [72] – where both auditory and somatosensory inputs are different – we expected different brain activities. However, comparison of high-pitched phonation and comfortable phonation did not show any significant activation in the brain. Possibly, the experimental paradigm did not allow to evaluate changes in brain activities during vocal pitch modulation. In our study, speech pathologist explained to all participants how to perform comfortable phonation tasks with a comfortable balance in the muscles of phonation and the respiratory system and, for high-pitched phonation tasks, to use maximal muscular activity. However, in order to reduce articulatory modifications that might cause artifacts during fMRI scanning we investigated rather unnatural phonation tasks, i.e. without labial and jaw movements. Moreover, an experimental paradigm involving phonation of the /i/ sound was opted for, in order to focus on sensorimotor integration control rather than motor control. A similar paradigm with phonation of /i/ was also used in the fMRI studies by Loucks et al [40] Haslinger et al [38], and Simonyan et al [73] to explore sensorimotor integration control during phonation and its disorders. An experimental paradigm involving phonation of the neutral sound /ə/ (schwa) at 3 vocal frequencies was chosen in the fMRI study by Peck et al [74] to evaluate laryngeal *motor* control of pitch modulation during phonation rather than laryngeal sensorimotor control. Their results showed activation in the bilateral cerebellum, left IFG, left cingulate gyrus, and left posterior cingulate during high pitch in comparison to comfortable pitch. The experimental paradigm with the phonation of /ə/ (schwa) sound was used to focus on laryngeal gestures [43, 44, 74]. For future research, in order to explore sensorimotor control in vocal pitch modulation, an experimental paradigm with phonation of the vowel /a/ instead of /i/ sound may be recommended to avoid F0 coinciding with the first formant (/i/ has a low F1 [300 Hz] whereas /a/ has a high F1 [800Hz]) [75].

The exhalation tasks in the present study also explored respiratory control. The fMRI data analysis showed a significant effect of exhalation control in the bilateral precentral gyrus, right lingual gyrus, left supramarginal gyrus, right thalamus, and cerebellum (table 3). In the fMRI study by Loucks et al [40], a comparable pattern of responses was identified for exhalation involving the left ventrolateral cortex, precentral and postcentral gyri, right supramarginal gyrus, right lingual gyrus, right cerebellum and

thalamus. In addition, the exhalation task allowed to generate laryngeal sensorimotor control maps by subtraction of the exhalation condition from the phonation condition [40, 56, 57]. Since the single cluster of differential activation in SFG was in the current study the only difference for the comfortable phonation and high-pitched phonation, these conditions were combined when comparing phonation (comfortable and high) and prolonged exhalation. This comparison revealed brain activity in the bilateral STG – the brain region associated with sound perception, postcentral gyrus – the brain region (S1) responsible for the proprioceptive sensation (proprioception) of the larynx, and in the precentral gyrus – the main laryngeal motor control center. In healthy female singers with supraglottic compression, brain activity in the pre/postcentral gyrus in addition to the STG may be a factor to recognize the neural mechanism that may affect laryngeal supraglotic compression during phonation. On one hand, this assumption is based on the conclusion of the fMRI study by Loucks et [40] and our previous fMRI study on brain activity associated with pitch modulation during phonation in healthy women without voice disorders [42]. These studies showed that neural control of exhalation for phonation is similar to neural control of voluntary exhalation, except for a difference in STG activation due to sensory feedback, particularly auditory feedback [40, 42]. On the other hand, this assumption is based on experiments by Kleber [76, 77] that provide evidence that singers rely more heavily on somatosensory feedback as a result of vocal training and practice. These fMRI studies on singing demonstrated that professional singers recruited more activity within S1 [76, 77]. In particular, singers are singing more accurately under anesthesia than non-musicians, despite the observed reduction of insular activity and functional connectivity [77].

The present pilot study also was used to fine-tune the experimental tasks and to identify problems with the experimental stimuli and/or procedures. The aim was to analyze brain activities in classically trained soprano singers with supralaryngeal compressions in three conditions: comfortable pitch, high pitch, and prolonged exhalation. The phonation tasks were not capable to show brain activation difference between high-pitched and comfortable phonation in participants. For future research, in order to explore vocal pitch modulation control as well as to avoid major resonance and articulatory changes, it may be recommended to use phonations of the vowel /a/ which has a first formant of 750-800Hz instead of /i/ which has a first formant of 300Hz in order to obtain F0 values lower than the first (expected) formant. Moreover, during the fMRI procedure there was no possibility to make audio recordings of phonations.

Therefore the actual difference in fundamental frequency between high pitch and comfortable pitch could not be determined. Before the fMRI scanning, however, an experienced speech therapist explained to all participants how to perform these tasks. Also, the participants of this study – trained singers – are used to perform vocal tasks with pitch modulation. For future research, we recommend using voice recordings within the fMRI setup. Furthermore, voice recording during stroboscopy is necessary to compare data.

The small number of subjects is a limitation of the present study. Furthermore, supraglottic activity was only studied in healthy female classical singers rather than other singing styles. Also, there is no comparison group of healthy female classical singers without supraglottic hyperactivity. For future research, comparison of the neural mechanism of phonation control in healthy singers with(out) laryngeal supraglotic compression in different singing styles could provide valuable information. The results of the present research may be used in the future to understand when supraglottic compression is dysfunctional, as in cases of functional dysphonia (such as MTD).

In summary, the neuroimaging data in this study reveal that healthy female singers with supraglottic compression use the bilateral pre/postcentral gyrus, frontal gyrus, cingulate gyrus, superior and middle temporal gyrus, parietal lobe, insula, lingual gyrus, cerebellum, thalamus, and brainstem for phonation control. Even with a small number of participants, we were able to locate brain regions important to phonation control [49, 66, 78-82], and to compare findings with the previous studies [42, 49, 66, 78-82]. The brain activity in the pre/postcentral gyrus in addition to the STG may be a factor to recognize the neural mechanism that may affect laryngeal supraglotic compression during phonation in healthy female singers. However, healthy female classical singers without supraglottic hyperactivity have to be included in future research as the best proof to understand impact of brain control on laryngeal behavior. We also suggest that the setup conditions of future fMRI experiments should be modified in order to make vocal pitch recording possible or to rely on fixed vocal pitches. An updated study protocol should provide further insight in the neural mechanisms of phonation related to laryngeal supraglotic compression in healthy speakers, in different singing styles, and in singers or speakers with voice problems, with the ultimate goal of understanding when laryngeal compression is either dysfunctional or normal.

## 5. Conclusions

The imaging results have demonstrated that healthy female classical singers with supraglottic compression use the auditory, motor, frontal, parietal, and subcortical areas to control phonation[46-48, 76, 77]. These areas are also used by other healthy people for the purpose of phonation control [83, 84]. However, subtracting the outcomes for neural control of voluntary exhalation from those of phonation revealed brain activities in the bilateral pre/postcentral areas and STG. In healthy female singers with supraglottic compression, the brain activity in the pre/postcentral gyrus may be a biomarker of laryngeal supraglottic compression during phonation. While the pilot study did not identify problems in the experimental stimuli, this study detected problems with the experimental procedures that necessitate refining the fMRI protocol. We suggest that the setup conditions of the future fMRI experiment should be changed in order to reduce unexpected effects and to use this protocol in future research on phonation and its disorders in order to understand when supraglottic compression is truly dysfunctional.

# 6.References


**1.** Wyke B. Reflexogenic contributions to vocal fold control systems. In: Titze IR, Ronald CS, eds. *Vocal fold physiology: biomechanics, acoustics and phonatory control* The Denver Center for the Performing Arts, Inc., Denver, Colorado: College-Hill; 1983:138-141.

**2.** Ludlow CL. Laryngeal Reflexes: Physiology, Technique, and Clinical Use. *Journal of Clinical Neurophysiology.* 2015;32:284-293.

**3.** Newman JD. *The physiological control of mammalian vocalization*: Springer Science & Business Media; 2012.

**4.** Ludlow CL. Central Nervous System Control of Voice and Swallowing. *Journal of Clinical Neurophysiology.* 2015;32:294-303.

**5.** Kelchner LN, Brehm SB, Weinrich B. *Pediatric Voice: A Modern, Collaborative Approach to Care*: Plural Publishing; 2014.

**6.** Chhetri DK, Neubauer J, Berry DA. Neuromuscular control of fundamental frequency and glottal posture at phonation onset. *The Journal of the Acoustical Society of America.* 2012;131:1401-1412.

**7.** Poletto CJ, Verdun LP, Strominger R, Ludlow CL. Correspondence between laryngeal vocal fold movement and muscle activity during speech and nonspeech gestures. *Journal of Applied Physiology.* 2004;97:858-866.

**8.** Ludlow CL. Central nervous system control of the laryngeal muscles in humans. *Respiratory physiology & neurobiology.* 2005;147:205-222.

**9.** Honda K, Hirai H, Masaki S, Shimada Y. Role of vertical larynx movement and cervical lordosis in F0 control. *Language and Speech.* 1999;42:401-411.

**10.** Kochis-Jennings KA, Finnegan EM, Hoffman HT, Jaiswal S. Laryngeal muscle activity and vocal fold adduction during chest, chestmix, headmix, and head registers in females. *Journal of Voice.* 2012;26:182-193.

**11.** Titze I. Principles of Voice ProductionPrentice Hall. *Englewood Cliffs, NJ.* 1994.

**12.** Hirano M, Ohala J. Use of hooked-wire electrodes for electromyography of the intrinsic laryngeal muscles. *Journal of Speech, Language, and Hearing Research.* 1969;12:362-373.

**13.** Verdolini-Marston K, Titze IR, Druker DG. Changes in phonation threshold pressure with induced conditions of hydration. *Journal of voice.* 1990;4:142-151.

**14.** Isshiki N. Vocal intensity and air flow rate. *Folia Phoniatrica et Logopaedica.* 1965;17:92-104.

**15.** Jones JA, Munhall KG. Perceptual calibration of F0 production: evidence from feedback perturbation. *The Journal of the Acoustical Society of America.* 2000;108:1246-1251.

**16.** Herbst CT, Hess M, Müller F, Švec JG, Sundberg J. Glottal adduction and subglottal pressure in singing. *Journal of Voice.* 2015;29:391-402.

**17.** Khoddami SM, Nakhostin Ansari N, Izadi F, Talebian Moghadam S. The assessment methods of laryngeal muscle activity in muscle tension dysphonia: a review. *The Scientific World Journal.* 2013;2013.

**18.** Angsuwarangsee T, Morrison M. Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice.* 2002;16:333-343.

**19.** Kooijman P, De Jong F, Oudes M, Huinck W, Van Acht H, Graamans K. Muscular tension and body posture in relation to voice handicap and voice quality in teachers with persistent voice complaints. *Folia phoniatrica et logopaedica.* 2005;57:134-147.

**20.** Lowell SY, Kelley RT, Colton RH, Smith PB, Portnoy JE. Position of the hyoid and larynx in people with muscle tension dysphonia. *The Laryngoscope.* 2012;122:370-377.

**21.** Hillman RE, Holmberg EB, Perkell JS, Walsh M, Vaughan C. Objective assessment of vocal hyperfunctionan experimental framework and initial results. *Journal of Speech, Language, and Hearing Research.* 1989;32:373-392.

**22.** Koufman J, Blalock P. Functional voice disorders. *Otolaryngologic Clinics of North America.* 1991;24:1059-1073.

**23.** Morrison M, Rammage L, Belisle G, Pullan C, Nichol H. Muscular tension dysphonia. *The Journal of otolaryngology.* 1983;12:302-306.

**24.** Roy N, Ford CN, Bless DM. Muscle tension dysphonia and spasmodic dysphonia: the role of manual laryngeal tension reduction in diagnosis and management. *Annals of Otology, Rhinology & Laryngology.* 1996;105:851-856.

**25.** Dworkin JP, Meleca RJ, Abkarian G. Muscle tension dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2000;8:169-173.

**26.** Stager SV, Bielamowicz SA, Regnell JR, Gupta A, Barkmeier JM. Supraglottic ActivityEvidence of Vocal Hyperfunction or Laryngeal Articulation? *Journal of speech, language, and hearing research.* 2000;43:229-238.

**27.** Behrman A, Dahl LD, Abramson AL, Schutte HK. Anterior-Posterior and medial compression of the supraglottis: signs of nonorganic dysphoniaor normal postures? *Journal of Voice.* 2003;17:403-410.

**28.** Morrison M, Rammage L. Muscle misuse voice disorders: description and classification. *Acta oto-laryngologica.* 1993;113:428-434.

**29.** Roy N. Functional dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2003;11:144-148.

**30.** Van Houtte E, Van Lierde K, D'haeseleer E, Claeys S. The prevalence of laryngeal pathology in a treatment□seeking population with dysphonia. *The Laryngoscope.* 2010;120:306-312.

**31.** Mayerhoff RM, Guzman M, Jackson□Menaldi C, et al. Analysis of supraglottic activity during vocalization in healthy singers. *The Laryngoscope.* 2014;124:504-509.

**32.** Guzman M, Ortega A, Olavarria C, et al. Comparison of supraglottic activity and spectral slope between theater actors and vocally untrained subjects. *Journal of Voice.* 2015.

**33.** Stager SV, Bielamowicz S, Gupta A, Marullo S, Regnell JR, Barkmeier J. Quantification of static and dynamic supraglottic activity. *Journal of Speech, Language, and Hearing Research.* 2001;44:1245-1256.

**34.** Sama A, Carding P, Price S, Kelly P, Wilson J. The clinical features of functional dysphonia. *The Laryngoscope.* 2001;111:458-463.

**35.** Lawrence V. Laryngological observations on belting. *J Res Singing.* 1979;2:26-28.

**36.** Yanagisawa E, Estill J, Kmucha ST, Leder SB. The contribution of aryepiglottic constriction to "ringing" voice quality—a videolaryngoscopic study with acoustic analysis. *Journal of Voice.* 1989;3:342-350.

**37.** Pershall KE, Boone DR. Supraglottic contribution to voice quality. *Journal of Voice.* 1987;1:186-190.

**38.** Haslinger B, Erhard P, Dresel C, Castrop F, Roettinger M, Ceballos-Baumann A. "Silent event-related" fMRI reveals reduced sensorimotor activation in laryngeal dystonia. *Neurology.* 2005;65:1562-1569.

**39.** Özdemir E, Norton A, Schlaug G. Shared and distinct neural correlates of singing and speaking. *Neuroimage.* 2006;33:628-635.

**40.** Loucks TM, Poletto CJ, Simonyan K, Reynolds CL, Ludlow CL. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. *Neuroimage.* 2007;36:131-143.

**41.** Murphy K, Corfield D, Guz A, et al. Cerebral areas associated with motor control of speech in humans. *Journal of Applied Physiology.* 1997;83:1438-1447.

**42.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2016.

**43.** Brown S, Laird AR, Pfordresher PQ, Thelen SM, Turkeltaub P, Liotti M. The somatotopy of speech: phonation and articulation in the human motor cortex. *Brain and cognition.* 2009;70:31-41.

**44.** Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cerebral Cortex.* 2008;18:837-845.

**45.** Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage.* 2006;31:1453-1474.

**46.** Perry DW, Zatorre RJ, Petrides M, Alivisatos B, Meyer E, Evans AC. Localization of cerebral activity during simple singing. *Neuroreport.* 1999;10:3979-3984.

**47.** Brown S, Martinez MJ, Hodges DA, Fox PT, Parsons LM. The song system of the human brain. *Cognitive Brain Research.* 2004;20:363-375.

**48.** Kleber B, Birbaumer N, Veit R, Trevorrow T, Lotze M. Overt and imagined singing of an Italian aria. *Neuroimage.* 2007;36:889-900.

**49.** Zarate JM, Zatorre RJ. Experience-dependent neural substrates involved in vocal pitch regulation during singing. *Neuroimage.* 2008;40:1871-1887.

**50.** Guzman M, Barros M, Espinoza F, et al. Laryngoscopic, acoustic, perceptual, and functional assessment of voice in rock singers. *Folia Phoniatrica et Logopaedica.* 2014;65:248-256.

**51.** Sakakibara K, Fuks L, Imagawa H, Tayama N, Naganuma D. Growl voice in ethnic and pop styles. *Proc. Int. Symp. on Musical Acoustics*2004.

**52.** Sakakibara K-I, Imagawa H, Konishi T, et al. Vocal fold and false vocal fold vibrations in throat singing and synthesis of khöömei. *Proceedings of the International Computer Music Conference, Havana, Cuba*: Citeseer; 2001:135-138.

**53.** Lindestad P-Å, Södersten M, Merker B, Granqvist S. Voice source characteristics in Mongolian "throat singing" studied with high-speed imaging technique, acoustic spectra, and inverse filtering. *Journal of Voice.* 2001;15:78-85.

**54.** Hamdan A-L, Sibai A, Moukarbel RV, Deeb R. Laryngeal biomechanics in Middle Eastern singing. *Journal of Voice.* 2006;20:579-584.

**55.** Guzman M, Lanas A, Olavarria C, et al. Laryngoscopic and spectral analysis of laryngeal and pharyngeal configuration in non-classical singing styles. *Journal of Voice.* 2015;29:130. e121-130. e128.

**56.** Miyapuram KP. Introduction to fMRI: experimental design and data analysis. 2008.

**57.** Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg M, Turner R. Event-related fMRI: characterizing differential responses. *Neuroimage.* 1998;7:30-40.

**58.** Rosen CA, Lee AS, Osborne J, Zullo T, Murry T. Development and validation of the Voice Handicap Index□10. *The Laryngoscope.* 2004;114:1549-1556.

**59.** Remacle M. The contribution of videostroboscopy in daily ENT practice. *Acta oto-rhino-laryngologica Belgica.* 1995;50:265-281.

**60.** Wuyts FL, De Bodt MS, Molenberghs G, et al. The Dysphonia Severity IndexAn Objective Measure of Vocal Quality Based on a Multiparameter Approach. *Journal of Speech, Language, and Hearing Research.* 2000;43:796-809.

**61.** Hirano M. Psycho-acoustic evaluation of voice: GRBAS scale. Clinical Examination of voice. Wien: Springer Verlag; 1981.

**62.** Hirano M. *Clinical examination of voice*: Springer; 1981.

**63.** Timmermans B, De Bodt MS, Wuyts FL, Van de Heyning PH. Analysis and evaluation of a voice-training program in future professional voice users. *Journal of Voice.* 2005;19:202-210.

**64.** Benninger MS, Murry T. *The singer's voice*: Plural Publishing; 2008.

**65.** Poburka BJ. A new stroboscopy rating form. *Journal of Voice.* 1999;13:403-413.

**66.** Flagmeier SG, Ray KL, Parkinson AL, et al. The neural changes in connectivity of the voice network during voice pitch perturbation. *Brain and language.* 2014;132:7-13.

**67.** Talairach J, Tournoux P. *Co-planar Stereotaxic Atlas of the Human Brain: 3-dimensional Proportional System : an Approach to Cerebral Imaging.* 1st ed. Stuggart, Germany: Thieme; 1988.

**68.** Calhoun VD, Liu J, Adalı T. A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. *Neuroimage.* 2009;45:S163-S172.

**69.** Bullmore E, Brammer M, Williams SC, et al. Statistical methods of estimation and inference for functional MR image analysis. *Magnetic Resonance in Medicine.* 1996;35:261-277.

**70.** Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage.* 2002;15:870-878.

**71.** Goldberg II, Harel M, Malach R. When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron.* 2006;50:329-339.

**72.** Sundberg J. The science of the singing voiceNorthern Illinois University Press. *Dekalb, IL.* 1987.

**73.** Simonyan K, Ludlow CL. Abnormal activation of the primary somatosensory cortex in spasmodic dysphonia: an fMRI study. *Cerebral Cortex.* 2010:bhp023.

**74.** Peck KK, Galgano JF, Branski RC, et al. Event-related functional MRI investigation of vocal pitch variation. *Neuroimage.* 2009;44:175-181.

**75.** Sundberg J. Formant technique in a professional female singer. *Acta Acustica united with Acustica.* 1975;32:89-96.

**76.** Kleber B, Veit R, Birbaumer N, Gruzelier J, Lotze M. The brain of opera singers: experience-dependent changes in functional activation. *Cerebral Cortex.* 2010;20:1144-1152.

**77.** Kleber B, Zeitouni AG, Friberg A, Zatorre RJ. Experience-dependent modulation of feedback integration during singing: role of the right anterior insula. *The Journal of Neuroscience.* 2013;33:6070-6080.

**78.** Toyomura A, Koyama S, Miyamaoto T, et al. Neural correlates of auditory feedback control in human. *Neuroscience.* 2007;146:499-503.

**79.** Parkinson AL, Flagmeier SG, Manes JL, Larson CR, Rogers B, Robin DA. Understanding the neural mechanisms involved in sensory control of voice production. *Neuroimage.* 2012;61:314-322.

**80.** Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. *Neuroimage.* 2008;39:1429-1443.

**81.** Parkinson AL, Korzyukov O, Larson CR, Litvak V, Robin DA. Modulation of effective connectivity during vocalization with perturbed auditory feedback. *Neuropsychologia.* 2013;51:1471-1480.

**82.** Zarate JM, Wood S, Zatorre RJ. Neural networks involved in voluntary and involuntary vocal pitch regulation in experienced singers. *Neuropsychologia.* 2010;48:607-618.

**83.** Kryshtopava M, Van Lierde K, Meerschman I, et al. Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders. *Journal of Voice.* 2017;31:118. e121-118. e128.

**84.** Kumar V, Croxson PL, Simonyan K. Structural organization of the laryngeal motor cortical network and its implication for evolution of speech production. *Journal of Neuroscience.* 2016;36:4170-4181.

# CHAPTER 6

# GENERAL CONCLUSIONS

## 1. General results and discussion

The pathophysiology of functional dysphonia is not fully understood [1-5]. The major pathophysiological finding in patients with functional voice disorders has been that the hyoid and larynx positions are higher in such patients than in controls [6]. Most recent studies have sought to recognize biomarkers of functional dysphonia. These studies have employed a variety of methods, such as paralaryngeal surface electromyography, mechanomyography, high-resolution manometry, and 24-h Dual-probe pH-metry [3-5, 7-12]. However, as yet no biomarkers or even any evidence for a primary biomechanical disturbance in patients with voice disorders have been reported in the literature; neither have significant differences in phonation-induced upper esophageal sphincter pressure or in electromyographic activity of submental, infrahyoidal, and sternocleidomastoid muscles between MTD patients and normal speakers been detected [3, 4, 13]. A better understanding of CNS control of voice might help to establish biomarkers and lead to the development of better diagnostic and treatment programs for patients with functional voice disorders. However, improvement of clinical outcomes in the treatment seeking population of professional voice users remains an important goal. The current study is an important step toward better understanding of the neural mechanisms underlying the brain activity of phonation in healthy women (with or without singing experience) and women with MTD. This study is the first in the field that has attempted to link routine voice diagnostic measures (i.e. perceptual, acoustic and aerodynamic) to brain imaging data in order to closely examine the relationship between current clinical voice measures and the underling neural mechanism of voice disorder in MTD. Information obtained from this study may enable the development of improved diagnostic and treatment programs for patients with functional voice disorders.

The ultimate goal of the research in both this study and the field in general is to explain the neurophysiological mechanism of laryngeal control of normal phonation and disordered phonation in functional voice disorders. In this regard, the VNS study focused on the VN as a possible channel for sensory stimuli relevant to phonation control. In fact, VNS served as a useful human model to illustrate the relevance of sensory stimuli on vocal changes and vocal control, which may be the same mechanisms underlying the symptoms of MTD patients. A series of fMRI studies of brain activity in healthy women (with or without singing experience) and in women with MTD were performed to establish a correlation

between brain reactivity and vocal tasks with the goal of determining neurological biomarkers that may define this pathological vocal behavior. Since neuroplastic change in the brain is an important mechanism that supports vocal learning and adaptation, sensory stimuli such as upper respiratory infection, second-hand smoke, LPR, significant vocal demands, or stressful life events might influence this neuroplasticity. We hypothesized that compared with healthy controls, MTD patients may have altered brain activities related to phonation control. Moreover, the authors hypothesized that the mechanism of vocal learning and adaptation[46] explains vocal hyperfunction during phonation in MTD patients. The implementation of neuroplasticity-based therapeutic training programs could be efficient in normalizing phonation and phonation control in MTD patients.

The goals of this study were: (1) to determine the vocal characteristics of patients treated with VNS in comparison with healthy controls; (2) to investigate brain activity during phonation in women with MTD in comparison with healthy controls using a specific fMRI protocol; and (3) to detect brain activity during phonation in healthy female singers with normal vocal characteristics and supraglottic compression using the specific fMRI protocol.

The first purpose was to determine the influence of VNS on vocal characteristics.

VNS has recently become an area of interest in the study of regulation of cortical plasticity[14, 15]. The VN contains 80% "afferent" (sensory) fibers carrying information from the body to the brain. Moreover, the larynx is mainly innervated by the SLN and the RLN from the VN. In the brainstem, the sensory afferent fibers terminate in the NTS, which then send fibers that connect directly or indirectly to different brain regions. These regions include the dorsal raphe nuclei, the locus coeruleus, the amygdala, the hypothalamus, the thalamus, the periaqueductal gray, the anterior cingulate cortex, and the orbitofrontal cortex. Previous fMRI studies have reported activation of these areas during VNS [16-33]. Many of these regions respond to and modulate phonation, e.g., the insula, the periaqueductal gray, the anterior cingulate cortex, the somatosensory cortices, the thalamus, and the prefrontal cortex. We evaluated 13 epileptic patients with implanted left VN stimulators. The main purpose of this study was to determine the objective and subjective vocal characteristics of patients treated with VNS. We hypothesized that, compared with

healthy controls, patients treated with VNS may have a significant decrease in objective vocal quality (decreased DSI value) together with disordered perceptual vocal characteristics and an impact of the voice disorder on QOL. Moreover, a significant correlation between the amount of stimulation and the presence of disturbed acoustic parameters was hypothesized.

Subjective (auditory-perceptual evaluation and VHI) and objective (aerodynamic, vocal range, and acoustic measurements and determination of the DSI) measurements were used to evaluate vocal quality in subjects with VNS under three different conditions (at rest and during normal and raised stimulation) and in the age- and gender-matched control group.

The VHI questionnaire revealed significant differences between the scores of the subjects with VNS and the control group in their self-perceived physical, functional, and emotional aspects of their vocal quality and on their QOL. However, the results of the subjects with VNS revealed the absence of an impact of their self-perceived vocal characteristics on their QOL. The absence of an impact of their self-perceived vocal characteristics on their QOL by means of the VHI score was not reflected in either the perceptual judgment (mean G2 R2 B1 A0 S0 I0) and the objective DSI value of – 2.4 (corresponding with a DSIof 26%). The auditory-perceptual impression of a moderate vocal disorder with the presence of a moderate roughness and slight breathiness was confirmed by the presence of a vocal quality value of – 2.4 in the subjects with VNS under resting conditions (no stimulation). A significant difference with the control group with a G0 R0 B0 A0 S0 I0, reflecting no perceptual vocal disorders, and DSI value of +3.5 (corresponding with a DSI of 85%) was measured. Analysis of the individual components of the DSI revealed that the primary variables responsible for the difference in DSI value between the subjects with and without VNS were the MPT (seconds), the jitter (%), and the F-high (Hz). The MPT is significantly shorter, the jitter percentage is significantly greater (reflecting more hoarseness), and the F-high is significantly lower in subjects with VNS.

Comparison of the acoustic parameters of the VNS and control voice samples under the three conditions revealed a significant difference in the F0; the higher the stimulation, the higher the F0 . Several different muscle actions are involved in raising vocal F0. The most important intrinsic laryngeal muscle

responsible for elongation of the vocal cords is the CT muscle. However, tension of the TA muscle and activity of the PCA muscle are also needed. The presence of complaints related to vocal change and QOL in patients undergoing VNS complaints may be caused by stimulation of the SLN and RLN and thereby excitation of either the afferent and/or efferent nerve fibers of the laryngeal system. Although these results should be viewed as preliminary, they suggest that the VN is a conduit for afferent and/or efferent signals that can influence phonation. Examination of the impact of VNS on the vocal characteristics enabled assessment of voice alteration by mimicking functional voice disorders that develop as a result of VNS and thereby, possibly, stimulating neural plasticity in the brain. Moreover, the results of this study have also informed a series of fMRI studies of brain activity associated with pitch adaptation during phonation in healthy women without voice disorders and in women with MTD.

The second purpose was to investigate brain activity during phonation in women with MTD in comparison with healthy controls using a specific fMRI protocol.

This study has proposed a blocked design fMRI experiment to investigate CNS control of voice with emphasis on laryngeal control of phonation. We implemented an experimental paradigm consisting of sustained phonation of the sound /i/ and prolonged exhalation tasks. The phonation tasks were designed to explore the interplay between respiratory and laryngeal control during phonation, whereas the exhalation tasks explored respiratory control separately. Additionally, the phonation tasks revealed the neural control associated with changes in respiratory and laryngeal adjustments to obtain vocal pitch modulations (comfortable and high). High pitch phonation produced at high intensity relies on maximal muscular activity of the intrinsic and extrinsic laryngeal muscles and the respiratory system. We hypothesized that a primary region related to the activation associated with vocal pitch (comfortable and high) adaptation/modulation would be the auditory cortex, more specifically the STG[34-40]. We also hypothesized that it would be observed by using the proposed fMRI protocol. We focused on the STG because it has been identified as an area of integration for sensory input and motor output during phonation[34, 35], specifically during error detection and correction involved in pitch processing[34, 36-39]. Furthermore, the STG is involved in auditory-vocal integration and processing of predicted and actual vocal output[40]. We investigated brain activity during phonation in sixteen healthy female subjects (mean age: 24.3 years, age

range: 21 – 28 years) without voice disorders using the proposed fMRI protocol. All steps of fMRI data preprocessing and analysis were performed using the BrainVoyager QX Version 2.4 software package (Brain Innovation, Maastricht, The Netherlands) [41]. Activation maps were generated by thresholding the statistical maps using the false discovery rate (FDR) approach ($q(FDR)<0.05$)[42].

This experiment relied on the identification of brain regions important to phonation and exhalation control [34, 37, 39, 41, 43-45]. Robust activation during phonation was observed in several of these regions, including the bilateral precentral gyrus, the SFG, the posterior cingulate gyrus, the STG, the MTG, the insula and cerebellum, the left IFG, the right cingulate, the lingual gyrus, and the thalamus. Several regions were also indicated during prolonged exhalation, including the bilateral precentral gyrus, the cerebellum, the right lingual gyrus, thethalamus, and the left supramarginal gyrus. Additionally, comparison of phonation (comfortable, high-pitched) tasks with prolonged exhalation tasks revealed activation in the bilateral STG and the insula. Activities in the bilateral STG and the right insula were greater in the case of high-pitched phonation, possibly reflecting a necessity for increased activity for the production of a higher frequency sound. Moreover, activity in the left midbrain PAG was present during the high pitch task, possibly reflecting a need for increased activity to process sensory input from higher vocal frequencies.

The results of this study showed that a blocked design fMRI paradigm is effective for the detection of a widespread set of cortical and sub-cortical regions associated with phonation and exhalation control in healthy people. Moreover, the results of this study enabled the implementation of the proposed blocked design fMRI experiment as a template for future research on the neural evaluation of phonation and its disorders.

To detect altered brain activity during phonation in women with MTD with the specific fMRI protocol, brain activity was investigated during phonation in women with MTD in comparison with healthy controls. We hypothesized that compared with healthy controls, MTD patients would have altered brain activities related to phonation control. Moreover, it was hypothesized that the mechanism of vocal learning and adaptation[46] explains vocal hyperfunction during phonation in MTD patients. Ten patients with MTD (mean age: 33,2 years, age range: 21 – 47 years) and fifteen healthy (mean age: 24.3 years, age range: 21 –

28 years) female, right-handed, native Flemish-speakers with no history of neurological or psychiatric disease participated in the study. All fMRI data preprocessing and fMRI data analysis (intragroup and intergroup) were performed using the BrainVoyager QX Version 2.4 software package (Brain Innovation, Maastricht, The Netherlands) [41]. Activation maps were generated by thresholding the statistical maps under the following conditions: P<0,001, 10 voxels, and uncorrected [47]. Comparison of two groups (MTD versus healthy) was performed using a "combined maps" approach (P<0.001, 10 voxels, uncorrected) and a subtraction approach[48-50] for fMRI data analysis of the comfortable phonation, high-pitched phonation and prolonged exhalation as well as for the comparisons between conditions of phonation and prolonged exhalation.

Brain activation during phonation was observed in the bilateral precentral gyrus, the right SFG, the MFG and IFG, the lingual gyrus, the cingulate gyrus, the STG, the thalamus (ventral posterior lateral nucleus), and the bilateral cerebellum in both groups. Brain activation during exhalation was observed in the bilateral precentral gyrus, the cingulate gyrus, the right lingual gyrus, and the bilateral cerebellum in both groups. These results are corroborated by an fMRI study by Loucks et al. [50].

Comparison of phonation (comfortable, high-pitched) tasks with prolonged exhalation tasks identified activation in the bilateral STG and insula in both groups. However, analysis of fMRI data from high-pitched phonation compared to comfortable phonation did not reveal any significant differences in brain activation for either group.

Comparison of phonation tasks in the two groups (MTD versus healthy) revealed *higher* brain activities during phonation (comfortable pitch, high-pitched) in the precentral gyrus, the inferior, middle and superior frontal gyrus, the lingual gyrus, the insula, the cerebellum, the midbrain, and in laryngeal motor control-related areas of the in the MTD group. Areas with *lower* activation during phonation (comfortable, high-pitched) were observed in the cingulate gyrus, the MTG and STG, and the inferior parietal lobe in the MTD group in comparison with healthy controls. No differences were observed between the two groups regarding exhalation control. Comparison of prolonged exhalation tasks in the two groups (MTD versus healthy) indicated a completely overlapping pattern of responses in the cerebral

regions mentioned above. Furthermore, comparison of phonation (comfortable and high-pitched) tasks with prolonged exhalation tasks in the two groups (MTD versus healthy) revealed areas with *higher* activation in the middle and superior frontal gyrus, and midbrain in the MTD group and areas with *lower* activation in the left middle temporal gyrus for comfortable phonation and in the right inferior parietal lobe for high-pitched phonation in the MTD group.

In patients with MTD, these altered (higher/lower) brain activities may result in laryngeal tension and voice symptoms. However, while this experiment was not able to provide evidence supporting internal representations/neural 'models' of the sensorimotor transformations changes, this experiment did provide evidence for altered neural correlates of phonation in MTD. In our study, altered neural activities were present during phonation in MTD patients in comparison with healthy controls, hypothetically supporting the theory that neural models provide a possible explanation for MTD and particularly for imbalanced laryngeal muscle activation in MTD. In MTD, abnormal sensory feedback (such as poor voice quality) may trigger the neural 'models' to stimulate new patterns of muscle activation and alter sensory perception (Figure 6.1). In particular, abnormal sensory feedback generates an error signal between the prediction of the sensory outcome of phonation and the incoming sensory feedback. The error signal updates the neural 'models' that in turn generate corrective commands to the motor controller and thus changes sensory perception (Figure 6.1). Altered descending motor cortical signals stimulate laryngeal motorneurons in the brainstem which might result in excessive tension [15]of the laryngeal muscles or recruit muscles that are not ordinarily active. Simultaneously, altered sensory perception makes the brain insensitive to the normal feedback even when irritants are no longer present. Thus, pathophysiology of MTD may be viewed as a processing of abnormal sensory information through an intact internal prediction/correction mechanism that results in the update or creation of new neural 'models'that alter muscle activation patterns and open sensory channels for abnormal sensory inputs. In this study, the lower neural activity in sensory control-related areas such as the STG, the MTG, and the inferior parietal lobe may reflect the suppression in these areas that makes the brain insensitive tonormal feedback. We also suggest that neuroplastic changes [51, 52] in the brain areas responsible to phonation control [53] may cause the symptoms of MTD. Furthermore, such updated neural 'models' generate corrective commands to the motor controller resulting in altered descending motor cortical signals. In our study, higher neural activity in the laryngeal motor control-related

areas such as the precentral gyrus, the SFG, the MFG, the IFG, the midbrain, the brainstem, and the cerebellum alters descending motor cortical signals and stimulates laryngeal motorneurons in the brainstem that may be responsible for laryngeal tension and voice symptoms in patients with MTD.

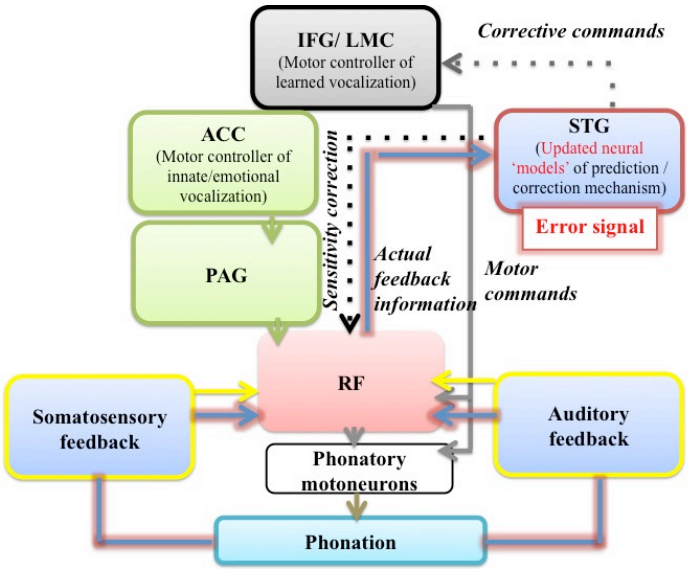


**Figure 6.1 Diagram of laryngeal neural control in muscle tension dysphonia** (modified from a neural model of vocalization proposed by Zarate[54]). In MTD, sensory stimulation associated with phonation is altered (indicated by red glowing arrows) and may trigger changes in the neural 'models': the mismatch between actual sensory information and prediction of the sensory outcome of motor commands (i.e. how it should feel) indicates an error signal (red glowing box). The error signal updates the neural 'models' that in turn generate corrective commands to the motor controller as well as alter sensory perception. The updated or new neural 'models' may support the symptoms of MTD by altering motor cortical commands in the areas responsible to motor control (e.g. the LMC, IFG) and changing sensory perception (changes in sensitivity) in the areas responsible to sensory control (e.g. the STG).

The third purpose was to detect brain activity during phonation in healthy female singers with supraglottic compression using the specific fMRI protocol.

Patients with vocal problems (e.g. functional dysphonia) often have limited pitch ranges due to laryngeal compression and postural problems during phonation [6, 55-57]. Moreover, recent studies have demonstrated that laryngeal compression may be a regular laryngeal behavior during normal speaking and singing [58-66]. However, to date no studies have been able to identify neural biomarkers that determine

supraglottic compression and help to understand whether laryngeal compression is either a dysfunctional or a normal laryngeal behavior.

This pilot study evaluated the feasibility for the use of fMRI to detect brain activation during phonation in healthy female singers with supraglottic compression. Four healthy female classical singers (mean age: 26 years, age range: 22-33) participated in the study. The average duration of voice training of the subjects was 8,25 years, with a range of 5-12 years. All steps of fMRI data preprocessing and fMRI group data analysis were performed using the BrainVoyager QX Version 2.4 software package (Brain Innovation, Maastricht, The Netherlands) [41]. Activation maps were generated by thresholding the statistical maps using the false discovery rate (FDR) approach ($q(FDR)<0.05$) [41, 42]. Group analysis indentified a significant primary effect of comfortable phonation in comparison with resting conditions in the right pre/postcentral gyrus, the left precentral gyrus, the bilateral middle frontal gyrus, the cingulate gyrus, the bilateral lingual gyrus, the bilateral STG extending to the inferior parietal lobe, the bilateral cerebellum, the brainstem. Significant effects of high-pitched phonation compared to resting conditions were also observed in the bilateral pre/postcentral gyrus, the bilateral superior frontal gyrus, the cingulate gyrus, the right lingual gyrus, the bilateral STG, the thalamus, the cerebellum, and the brainstem. Increased activity in the bilateral SFG was observed during high-pitched phonation in comparison to the rest period. Group analyses revealed a significant primary effect of exhalation compared to the rest period in the bilateral precentral gyrus, the right lingual gyrus, the left supramarginal gyrus, the right thalamus, and the cerebellum. Comparing phonation (comfortable and high) and prolonged exhalation tasks revealed a significant primary effect of phonation in the bilateral pre/postcentral gyrus and the STG.

The results of these imaging studies have demonstrated that healthy female classical singers with supraglottic compression use the auditory, motor, frontal, parietal, and subcortical areas to control phonation. These areas are also used by other healthy people for the purpose of phonation control. Subtracting the outcomes for neural control of voluntary exhalation from those of phonation revealed significant brain activity in the bilateral pre/postcentral areas and the STG. In healthy female singers with supraglottic compression, brain activity in the pre/postcentral gyrus may be a biomarker of laryngeal supraglottic compression during phonation.

## 2. Strengths and limitations of the study

This study of the impact of VNS on vocal characteristics has demonstrated that subjects with VNS had a disordered perceptual and objective vocal quality. During stimulation and especially during raised stimulation, the fundamental frequency was significantly increased. This is an example of the influence of neurological perturbation on vocal output. However, a limitation of the study is the absence of laryngeal videostroboscopic recordings that correlate with the perceptual characteristics and DSI values that were recorded during the investigation of the impact of VNS on the objective and subjective vocal characteristics. The use of laryngeal videostroboscopic evaluation was not possible in this vulnerable population. Also, a longitudinal study design (following the same subject before and several times after implantation) would have been a better choice but was not possible because of practical reasons.

The study of CNS control of voice in healthy women and women with MTD has demonstrated that MTD patients use the auditory, motor, frontal, parietal, and subcortical areas for phonation control. Healthy people for the purpose of phonation control also use these brain areas. However, comparison of phonation tasks in the two groups (MTD versus healthy) revealed *higher* brain activities in the precentral gyrus, the inferior, middle and superior frontal gyrus, the lingual gyrus, the insula, the cerebellum, the midbrain, and the brainstem as well as *lower* brain activities in the cingulate gyrus, the superior and middle temporal gyrus, and the inferior parietal lobe in the MTD group. In patients with MTD, these altered (higher/lower) brain activities may result in laryngeal tension and voice symptoms.

In our experiment, activity in the bilateral SFG was present during the high-pitch phonation task in both groups (MTD and healthy). We may only hypothesize that the reaction of the brain to external or internal stimuli may vary according to individual personality characteristics and/or behavior. Goldberg reported that when a personal emotional response was required, participants showed activity in the SFG – the brain region associated with self-awareness-related function[67]. In our experiment, activity in the bilateral SFG was present during the high-pitch phonation task, hypothetically reflecting greater emotional activity co-occurring with the greater vocal effort required to control high-pitched phonation. Moreover, the brain responds to environmental circumstances by creating neural circuits as needed (for example to

facilitate new learning or to cope with environmental stressors) and by pruning the neural pathways that are no longer used. Neural circuits are bolstered when people practice a new skill or new ways of reacting to a situation – the neural circuits become "hardwired" or preprogrammed into the brain. This is true for healthy, productive thoughts and behaviors, as well as for distressing or dysfunctional thoughts and behaviors. Future studies will be required in order to learn more about human brain activity in response to environmental (external) or systemic (internal) stimuli (such as motivational, affective, behavioral, and environmental conditions) and to examine the possible correlation between the behavior andpersonality types of patients with MTD and the pathophysiology of MTD.

This study of CNS control of voice in healthy female singers with supraglottic compression has demonstrated a significant effect of phonation control in the bilateral pre/postcentral gyrus, and in the frontal, cingulate, superior and middle temporal gyrus, as well as in the parietal lobe, the insula, the lingual gyrus, the cerebellum, the thalamus, and the brainstem. These activation areas are consistent with previous reports using other fMRI protocols. In addition, a significant effect of phonation was found in the bilateral superior temporal gyrus, and the pre/postcentral gyrus. In healthy female singers with supraglottic compression, the brain activity in the pre/postcentral gyrus may be a biomarker of laryngeal supraglottic compression during phonation.

The results of this study were also was employed to fine-tune the experimental design and to identify problems with the experimental stimuli and/or procedures. The fMRI study investigated CNS control of voice in healthy women, women with MTD, and healthy female singers with supraglottic compression under three conditions: comfortable pitch, high pitch, and prolonged exhalation. However, during the fMRI procedure there was no possibility to make audio recordings of phonations. Therefore the actual difference in F0 between high pitch and comfortable pitch could not be determined. In future research, we recommend the collection of voice recordings within the fMRI setup. Furthermore, voice recording during stroboscopy is necessary for the comparison of experimental data. Moreover, the phonation tasks did not reveal differences in brain activation between high-pitched and comfortable phonation in MTD patients and control subjects. In order to explore vocal pitch modulation control as well as to avoid major resonance and articulatory changes, the use of additional phonations of the vowel /a/ (first

resonance of 750Hz) are indicated instead of /i/ in order to obtain F0 values lower than the first (expected) resonance[68].

Moreover, fMRI measurements are particularly sensitive to motion artifacts. There are a number of sources of motion in fMRI experiments that may result in image artifacts, such as jaw and tongue motion, swallowing, and oral and pharyngeal muscle activity [69]. These artifacts are extremely difficult to remove during fMRI data preprocessing. The development of event related sparse sampling was a significant technological advance allowing the use of fMRI for the study of speech, voice, and swallowing [70]. Sparse sampling fMRI is used for the presentation of auditory stimuli without contamination by acoustic scanner noise and for overt vocal responses without motion-induced artifacts in the functional time series. In the study performed by Hall et al.[71] it was reported that the mean peak of response in sparse sampling fMRI was in 10.5 seconds after the stimulus onset, and that the mean latency of the haemodynamic maximum and minimum was 7.7 seconds and 8.1 seconds after stimulus onset and offset, respectively. In our study we used a blocked design with a continuous acquisition fMRI method of examination, which may also be viewed as a limitation of the study. We had no possibility to use sparse sampling fMRI due to a special protocol settings adapted to MTD patients. In our study, we used continuous fMRI as no auditory stimuli were used in the experiment. Moreover, the mean peak of response was 5-6 seconds (not 10 sec as in sparse sampling fMRI) after the stimulus onset. In our experiment, each of experimental tasks (phonation and exhalation) was explained to produce with the same oral posture/condition (with minimal jaw movements and oral muscles activity). The subjects underwent a 30-min training session prior to the scanning session in order to learn how to perform the tasks in a highly controlled manner with a minimum of head, lip, jaw, tongue, and body movement during the testing. Before the 30-min training session, the subjects were instructed how to perform tasks by an experienced speech therapist. The subjects were taught to keep their jaw slightly open and to keep their jaw, lips, and tongue motionless to minimize oral movements during all tasks.

The monitoring of the execution of the tasks during fMRI was performed without an analysis of the voice samples and may be regarded as a limitation of the fMRI study. The audio systems in the fMRI recordings did not provide recording of voice samples. The project leader (S.C.) and MRI operator (M.K.)

monitored the execution of the tasks throughout the study through a control room speaker to assure that participants produced phonation tasks correctly. In addition, the noise cancelling MRI headphones and a noise cancelling microphone provided the highest level of fMRI noise reduction possible, allowing participants to self-monitor and adjust their tasks production. Moreover, participants were evaluated by the same experienced speech therapist, samples of voice based on the production of a sustained vowel /i/ were recorded and F0 for each subject was assessed.

The fMRI method of examination cannot be routinely used at all hospitals since they requires a special diagnostic system, are complex, and expensive, and may be regarded as a limitation of the implementation in clinical practice of the fMRI study.

## 3. Suggestions for future research

Information obtained from this study may allow the development of improved diagnostic and treatment programs for patients with functional voice disorders. Based on the results of this study, future research should focus on the following main research directions.

The first direction is the investigation of the impact of VNS on the intrinsic laryngeal muscles responsible for the increase of the vocal pitch. Comparison of the vocal characteristics of subjects with refractory epilepsy with and without VNS would have provided valuable information for the fMRI studies and is a subject for further research.

The second direction for future research would be study of the central neuroplastic change mechanisms. The central neuroplastic change mechanisms can be measured by using electrophysiological recording methods, the auditory evoked potentials (AEPs). Brainstem AEPs to *speech* sounds are used as a biological marker of deficient auditory processing associated with language and learning disorders and mechanisms of plasticity in the auditory brainstem. However this method of examination cannot be routinely used for voice disorders as it requires modifications and the special diagnostic system of the fMRI to be applied to the study of voice. More affordable methods for study of abnormailities in the sensory gating mechanismin voice disorders must be proposed. It may be possible to explain the relationship

between current clinical voice measures and the underling neural mechanism of voice control and its disorder in dysphonia by linking routine voice diagnostic measures (i.e. perceptual, acoustic and aerodynamic) to a decrease in habituation of the P50 potentials and amplitude and latency of the P300 in patients with dysphonia.. From this perspective, AEPs may be used as a biological marker of deficient auditory processing associated with voice disorders and mechanisms of plasticity in the brain.

Novel treatment programs for patients with functional voice disorders must be developed. A target of these programs includes the neuroplastic changes in the brain that underlie vocal learning and adaptation. We assume that plasticity-driven changes, if appropriately implemented, will significantly renormalize the brain system in ways that reestablish vocal behavior. For the development of neuroplasticy-based therapeutic training programs, in our study we have attempted to investigate brain activity during phonation and to describe specific biomarkers in MTD patients.

In conclusion, future research in voice disorders should be focused on the study of brain regions using functional imaging techniques and electrophysiological recording methods. Functional and structural MRI methods have developed considerably in recent decades, and they may now be applied to study patients with voice disorders. Electrophysiological recording methods must be modified for voice diagnostics and may help to determine biomarkers of neuroplasticity in developing vocal skills and voice disorders as well as sensory gating disorders. An interdisciplinary study group including neurologists, otolaryngologists and speech pathologists, as well as experts in experimental design and statistical analysis will need to conduct and assess these investigations. Moreover, in therapeutic programs for patients with functional voice disorders, treatment strategies that result in renormalizing learning-control mechanisms of the brain should to be developed.

## 4. References


**1.** Roy N. Functional dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2003;11:144-148.

**2.** Dworkin JP, Meleca RJ, Abkarian G. Muscle tension dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery.* 2000;8:169-173.

**3.** Van Houtte E, Claeys S, D'haeseleer E, Wuyts F, Van Lierde K. An examination of surface EMG for the assessment of muscle tension dysphonia. *Journal of Voice.* 2013;27:177-186.

**4.** Van Houtte E, Van Lierde K, D'haeseleer E, Van Imschoot B, Claeys S. UES Pressure During Phonation Using High-resolution Manometry and 24-h Dual-probe pH-metry in Patients with Muscle Tension Dysphonia. *Dysphagia.* 2012;27:198-209.

**5.** Van Houtte E, Van Lierde K, Claeys S. Pathophysiology and treatment of muscle tension dysphonia: a review of the current knowledge. *Journal of Voice.* 2011;25:202-207.

**6.** Lowell SY, Kelley RT, Colton RH, Smith PB, Portnoy JE. Position of the hyoid and larynx in people with muscle tension dysphonia. *The Laryngoscope.* 2012;122:370-377.

**7.** Hocevar-Boltezar I, Janko M, Zargi M. Role of surface EMG in diagnostics and treatment of muscle tension dysphonia. *Acta Otolaryngologica.* 1998;118:739-743.

**8.** Redenbaugh MA, Reich AR. Surface EMG and related measures in normal and vocally hyperfunctional speakers. *Journal of Speech and Hearing Disorders.* 1989;54:68-73.

**9.** Stepp CE, Heaton JT, Braden MN, Jette ME, Stadelman-Cohen TK, Hillman RE. Comparison of neck tension palpation rating systems with surface electromyographic and acoustic measures in vocal hyperfunction. *Journal of Voice.* 2011;25:67-75.

**10.** Stepp CE, Heaton JT, Jetté ME, Burns JA, Hillman RE. Neck surface electromyography as a measure of vocal hyperfunction before and after injection laryngoplasty. *The Annals of otology, rhinology, and laryngology.* 2010;119:594.

**11.** Stepp CE, Heaton JT, Stadelman-Cohen TK, Braden MN, Jetté ME, Hillman RE. Characteristics of phonatory function in singers and nonsingers with vocal fold nodules. *Journal of voice.* 2011;25:714-724.

**12.** Stepp CE, Hillman RE, Heaton JT. Use of neck strap muscle intermuscular coherence as an indicator of vocal hyperfunction. *Neural Systems and Rehabilitation Engineering, IEEE Transactions on.* 2010;18:329-335.

**13.** Bove M, Ruth M, Cange L, Månsson I. 24-H pharyngeal pH monitoring in healthy volunteers: a normative study. *Scandinavian journal of gastroenterology.* 2000;35:234-241.

**14.** Hays SA, Rennaker RL, Kilgard MP. Targeting plasticity with VN stimulation to treat neurological disease. *Progress in brain research.* 2013;207:275.

**15.** Porter BA, Khodaparast N, Fayyaz T, et al. Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. *Cerebral Cortex.* 2011;22:2365-2374.

**16.** Narayanan JT, Watts R, Haddad N, Labar DR, Li PM, Filippi CG. Cerebral activation during vagus nerve stimulation: a functional MR study. *Epilepsia.* 2002;43:1509-1514.

**17.** Van Laere K, Vonck K, Boon P, Brans B, Vandekerckhove T, Dierckx R. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. *Journal of Nuclear Medicine.* 2000;41:1145-1154.

**18.** Van Laere K, Vonck K, Boon P, Versijpt J, Dierckx R. Perfusion SPECT changes after acute and chronic vagus nerve stimulation in relation to prestimulus condition and long-term clinical efficacy. *Journal of Nuclear Medicine.* 2002;43:733-744.

**19.** Yakunina N, Kim SS, Nam EC. Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation: Technology at the Neural Interface.* 2017;20:290-300.

**20.** Bohning DE, Lomarev MP, Denslow S, Nahas Z, Shastri A, George MS. Feasibility of vagus nerve stimulation–synchronized blood oxygenation level–dependent functional MRI. *Investigative Radiology.* 2001;36:470-479.

**21.** Ring H, White S, Costa D, et al. A SPECT study of the effect of vagal nerve stimulation on thalamic activity in patients with epilepsy. *Seizure.* 2000;9:380-384.

**22.** Vonck K, Boon P, Laere K, et al. Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. *Epilepsia.* 2000;41:601-609.

**23.** Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. *Seizure.* 2008;17:699-706.

**24.** Vonck K, Van Laere K, Dedeurwaerdere S, Caemaert J, De Reuck J, Boon P. The mechanism of action of vagus nerve stimulation for refractory epilepsy: the current status. *Journal of clinical neurophysiology.* 2001;18:394-401.

**25.** Lomarev M, Denslow S, Nahas Z, Chae J-H, George MS, Bohning DE. Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects. *Journal of psychiatric research.* 2002;36:219-227.

**26.** Liu WC, Mosier K, Kalnin A, Marks D. BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. *Journal of Neurology, Neurosurgery & Psychiatry.* 2003;74:811-813.

**27.** Nahas Z, Teneback C, Jeong-Ho C, et al. Serial vagus nerve stimulation functional MRI in treatment-resistant depression. *Neuropsychopharmacology.* 2007;32:1649.

**28.** Dietrich S, Smith J, Scherzinger C, et al. A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI/Funktionelle Magnetresonanztomographie zeigt Aktivierungen des Hirnstamms und weiterer zerebraler Strukturen unter transkutaner Vagusnervstimulation. *Biomedizinische Technik/Biomedical Engineering.* 2008;53:104-111.

**29.** Kraus T, Hösl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of neural transmission.* 2007;114:1485-1493.

**30.** Kraus T, Kiess O, Hösl K, Terekhin P, Kornhuber J, Forster C. CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal–a pilot study. *Brain stimulation.* 2013;6:798-804.

**31.** Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain stimulation.* 2015;8:624-636.

**32.** Frangos E, Komisaruk BR. Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. *Brain stimulation.* 2017;10:19-27.

**33.** Frangos E, Richards EA, Bushnell MC. Do the psychological effects of vagus nerve stimulation partially mediate vagal pain modulation? *Neurobiology of Pain.* 2017.

**34.** Parkinson AL, Flagmeier SG, Manes JL, Larson CR, Rogers B, Robin DA. Understanding the neural mechanisms involved in sensory control of voice production. *Neuroimage.* 2012;61:314-322.

**35.** Hickok G, Houde J, Rong F. Sensorimotor integration in speech processing: computational basis and neural organization. *Neuron.* 2011;69:407-422.

**36.** Behroozmand R, Larson CR. Error-dependent modulation of speech-induced auditory suppression for pitch-shifted voice feedback. *BMC neuroscience.* 2011;12:54.

**37.** Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. *Neuroimage.* 2008;39:1429-1443.

**38.** Guenther FH, Ghosh SS, Tourville JA. Neural modeling and imaging of the cortical interactions underlying syllable production. *Brain and language.* 2006;96:280-301.

**39.** Zarate JM, Zatorre RJ. Experience-dependent neural substrates involved in vocal pitch regulation during singing. *Neuroimage.* 2008;40:1871-1887.

**40.** Zarate JM, Zatorre RJ. Neural substrates governing audiovocal integration for vocal pitch regulation in singing. *Annals of the New York Academy of Sciences.* 2005;1060:404-408.

**41.** Flagmeier SG, Ray KL, Parkinson AL, et al. The neural changes in connectivity of the voice network during voice pitch perturbation. *Brain and language.* 2014;132:7-13.

**42.** Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage.* 2002;15:870-878.

**43.** Toyomura A, Koyama S, Miyamaoto T, et al. Neural correlates of auditory feedback control in human. *Neuroscience.* 2007;146:499-503.

**44.** Parkinson AL, Korzyukov O, Larson CR, Litvak V, Robin DA. Modulation of effective connectivity during vocalization with perturbed auditory feedback. *Neuropsychologia.* 2013;51:1471-1480.

**45.** Zarate JM, Wood S, Zatorre RJ. Neural networks involved in voluntary and involuntary vocal pitch regulation in experienced singers. *Neuropsychologia.* 2010;48:607-618.

**46.** Urberg-Carlson KE. *Muscle tension dysphonia as a disorder of motor learning*, University of Minnesota; 2013.

**47.** Lieberman MD, Cunningham WA. Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social cognitive and affective neuroscience.* 2009:nsp052.

**48.** Miyapuram KP. Introduction to fMRI: experimental design and data analysis. 2008.

**49.** Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg M, Turner R. Event-related fMRI: characterizing differential responses. *Neuroimage.* 1998;7:30-40.

**50.** Loucks TM, Poletto CJ, Simonyan K, Reynolds CL, Ludlow CL. Human brain activation during phonation and exhalation: common volitional control for two upper airway functions. *Neuroimage.* 2007;36:131-143.

**51.** Malandraki GA, Johnson S, Robbins J. Functional MRI of swallowing: from neurophysiology to neuroplasticity. *Head & neck.* 2011;33:S14-S20.

**52.** Kleim JA, Jones TA. Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *Journal of speech, language, and hearing research.* 2008;51:S225-S239.

**53.** Pynn LK, DeSouza JF. The function of efference copy signals: implications for symptoms of schizophrenia. *Vision research.* 2013;76:124-133.

**54.** Zarate JM. The neural control of singing. *Frontiers in human neuroscience.* 2013;7.

**55.** Khoddami SM, Nakhostin Ansari N, Izadi F, Talebian Moghadam S. The assessment methods of laryngeal muscle activity in muscle tension dysphonia: a review. *The Scientific World Journal.* 2013;2013.

**56.** Angsuwarangsee T, Morrison M. Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice.* 2002;16:333-343.

**57.** Kooijman P, De Jong F, Oudes M, Huinck W, Van Acht H, Graamans K. Muscular tension and body posture in relation to voice handicap and voice quality in teachers with persistent voice complaints. *Folia phoniatrica et logopaedica.* 2005;57:134-147.

**58.** Mayerhoff RM, Guzman M, Jackson□Menaldi C, et al. Analysis of supraglottic activity during vocalization in healthy singers. *The Laryngoscope.* 2014;124:504-509.

**59.** Guzman M, Ortega A, Olavarria C, et al. Comparison of supraglottic activity and spectral slope between theater actors and vocally untrained subjects. *Journal of Voice.* 2015.

**60.** Stager SV, Bielamowicz SA, Regnell JR, Gupta A, Barkmeier JM. Supraglottic ActivityEvidence of Vocal Hyperfunction or Laryngeal Articulation? *Journal of speech, language, and hearing research.* 2000;43:229-238.

**61.** Behrman A, Dahl LD, Abramson AL, Schutte HK. Anterior-Posterior and medial compression of the supraglottis: signs of nonorganic dysphoniaor normal postures? *Journal of Voice.* 2003;17:403-410.

**62.** Stager SV, Bielamowicz S, Gupta A, Marullo S, Regnell JR, Barkmeier J. Quantification of static and dynamic supraglottic activity. *Journal of Speech, Language, and Hearing Research.* 2001;44:1245-1256.

**63.** Sama A, Carding P, Price S, Kelly P, Wilson J. The clinical features of functional dysphonia. *The Laryngoscope.* 2001;111:458-463.

**64.** Lawrence V. Laryngological observations on belting. *J Res Singing.* 1979;2:26-28.

**65.** Yanagisawa E, Estill J, Kmucha ST, Leder SB. The contribution of aryepiglottic constriction to "ringing" voice quality—a videolaryngoscopic study with acoustic analysis. *Journal of Voice.* 1989;3:342-350.

**66.** Pershall KE, Boone DR. Supraglottic contribution to voice quality. *Journal of Voice.* 1987;1:186-190.

**67.** Goldberg II, Harel M, Malach R. When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron.* 2006;50:329-339.

**68.** Sundberg J. Formant technique in a professional female singer. *Acta Acustica united with Acustica.* 1975;32:89-96.

**69.** Birn RM, Bandettini PA, Cox RW, Jesmanowicz A, Shaker R. Magnetic field changes in the human brain due to swallowing or speaking. *Magnetic Resonance in Medicine.* 1998;40:55-60.

**70.** Birn RM, Bandettini PA, Cox RW, Shaker R. Event-related fMRI of tasks involving brief motion. *Human brain mapping.* 1999;7:106-114.

**71.** Hall DA, Haggard MP, Akeroyd MA, et al. Sparse temporal sampling in auditory fMRI. *Human brain mapping.* 1999;7:213-223.

# CURRICULUM VITAE


**Name** **KRYSHTOPAVA**

**First name** **MARYNA**

**Work address** Department of Otorhinolaryngology,
Ghent University Hospital,
De Pintelaan, 185 1P1, 9000 Ghent, Belgium
**E-mail:** Maryna.Kryshtopava@ugent.be
**Phone:** +32 933221247


**Education**

| | | |
|---|---|---|
| 2013 – present | Ph.D. candidate | Thesis "***Functional magnetic resonance imaging study of central neural system control of voice, with emphasis on phonation in healthy women and women with muscle tension dysphonia***"<br>Tutor: Professor S.Claeys<br>Faculty of Medicine and Health Sciences,<br>Department of Otorhinolaryngology,<br>Ghent University, Belgium |
| 2004 – 2008 | Candidate of Medical Sciences<br>GPA: 5.0/5.0 | Thesis **"*A method of septoplasty using a synthetic mesh implant*"**<br>Tutor: Professor V. Kunitsky<br>Department of Otorhinolaryngology,<br>Vitebsk State Medical University, Belarus |
| 2002 – 2007 | Senior Fellow, Fellow, Resident | Specialization: Otorhinolaryngology<br>Department of Otorhinolaryngology,<br>Vitebsk State Medical University, Belarus |
| 1996-2002 | M.D. (with the highest honors) | Vitebsk State Medical University, Belarus |

**Major publications**

K. Van Lierde*, **M. Kryshtopava*,** G. Van Maele, B. Boehme, N. Piens, K. Vonck "Impact of vagal nerve stimulation on objective vocal quality, a pilot study" **Journal of Voice**, 29.6 (2015): 777-e9.
*equal contribution

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, P. Vandemaele, G. Vingerhoets, S. Claeys "Functional Magnetic Resonance Imaging Study of Brain Activity Associated With Pitch Adaptation During Phonation in Healthy Women Without Voice Disorders"**. Journal of Voice**;31:118 (2017): e121-118. e128.

D'haeseleer E., Behlau M., Bruneel L., Meerschman I., Luyten A., Lambrecht S., Cassol M., Corthals P., **Kryshtopava M.,** Wuyts F.L., Claeys S., Van Lierde K. "Factors Involved in Vocal Fatigue: A Pilot Study". **Folia Phoniatrica et Logopaedica**, 68 (2016):112-118.

**Kryshtopava M.,** Van Lierde K., Meerschman I., Vandemaele P., Vingerhoets G., and Claeys S. "Brain activity during phonation in women with muscle tension dysphonia: an fMRI study". **Journal of Voice** (in press).

**Kryshtopava M.,** Van Lierde K., Defrancq C.; De Moor M.; Thijs Z.; Meerschman I., Vandemaele P., Vingerhoets G., and Claeys S. Brain activity during phonation in healthy female singers with supraglottic hyperfunction: an fMRI pilot study (submitted to Journal of Logopedics Phoniatrics Vocology in November, 2016)

**Kryshtopava M.,** Van Lierde K., Claeys S. "Laryngeal neural control of phonation and functional voice disorders" (in progress)

**Oral presentations**

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, P. Vandemaele, G. Vingerhoets, S. Claeys "Neurophysiological foundation of human phonation: a pilot fMRI study". Oral presentation. The Third Polish Lithuanian ENT Congress on September 26, 2015, Augustow (Poland)

K. Van Lierde, **M. Krystopava,** G. Van Maele, B. Boehme, N. Piens, K. Vonck "Impact of Nervus vagus stimulation on objective vocal quality, a pilot study". Oral presentation. Care of the professional voice, 27-31 05/2015, Philadelphia (USA).

**M. Kryshtopava,** K. Van Lierde, S. Claeys How talented is your voice? Oral presentation. Science Day, November, 2015, Ghent (Belgium).
**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, P. Vandemaele, G. Vingerhoets, S. Claeys "Brain Mapping of Laryngeal Sensorimotor Control in Normal Phonation" Oral presentation. The 10th International Conference on Voice Physiology and Biomechanics, March 14 - 17, 2016, Viña del Mar (Chile)

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, S. Claeys "Functional magnetic resonance imaging study of brain activity during phonation in women with functional voice disorders" Oral presentation, Research Day, 16.03.2016, Ghent (Belgium)

I. Meerschman, E. D"Haeseleer, S. De Ley, **M. Krystopava,** S. Claeys, K.Van Lierde "Muscle Tension Dysphonia" Oral presentation. Symposium 'Voice and Muscle Tension' 16/04/2016, Brugge (Belgium)

**M. Kryshtopava**, M. De Moor, E. D'Haeseleer, K. Van Lierde, S. Claeys "Brain activity during phonation in healthy women with singing experience: an fMRI pilot study" Poster presentation. Studenten OnderzoeksSymposium (SOS), 26.04.2016, Ghent (Belgium) – Best poster presentation award!

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, P. Vandemaele, G. Vingerhoets, S. Claeys "Functional Magnetic Resonance Imaging Study of Brain Activity During Phonation In Women With Muscle Tension Dysphonia" Poster presentation, Knowledge for Growth, 6 May 2016 Ghent (Belgium).

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, G. Vingerhoets, S. Claeys "Central Neural Correlates Of Laryngeal Tension During Phonation In Muscle Tension Dysphonia: A Functional Magnetic Resonance Imaging Study" Oral presentation, 12th Salzburg Voice Symposium, brain & voice, 26 – 28 August, 2016 (Austria).

De Francq C.; De Moor M.; Thijs Z.; **Kryshtopava M.**; D'Haeseleer E.; Meerschman I.; Van Lierde K.; Vingerhoets G.; Claeys S. "Brain activity during phonation in healthy women with singing experience: an fMRI pilot study " Oral presentation, 12th Salzburg Voice Symposium, brain & voice, 26 – 28 August, 2016 (Austria).

K. Van Lierde, **M. Krystopava,** S. Gadeyne, A. Luyten, E. D'haseleer, L. Bruneel, G. Van Maele, B. Boehme, N. Piens, K. Vonck Impact of vagal nerve stimulation on objective vocal quality: a pilot study" Oral presentation 24 august Dublin, 30th World Congress of the IALP

K. Van Lierde, M. Behlau, E. Dhaeseleer, L. Bruneel, I. Meerschman, A. Luyten, S. lembrecht, M. Cassol, P. Corthals, **M. Krystopava,** F. Wuytens, S. Claeys "Factors involved in vocal fatique: a pilot study" Oral presentation 24 august Dublin, 30th World Congress of the IALP

**M. Kryshtopava,** K. Van Lierde, I. Meerschman, E. D'Haeseleer, G. Vingerhoets, S. Claeys "Functional magnetic resonance imaging study of central neural system control of voice, with emphasis on phonation in women with muscle tension dysphonia". Oral presentation, Royal Belgian Society for Ear, Nose, Throat, Head and Neck Surgery Spring Symposium 25 March 2017, Brussels, Belgium

**Patents**

- *"Device for obtaining cartilage tissue layer slices"* (Belarus #3137, 2006);
- *"Implant"* (Belarus # 3938, 2007);
- *"A method for the reconstruction of the deformed nasal septum"* (Belarus #12702; 2009);
- *"The method of surgical treatment of the deformation of the nasal septum"* (Belarus #12819, 2010);

**Clinical Trails And Implementation In Practice For Developed Materials And Methods**

- *"A polymer-based bio-engineered material for reconstruction of cartilage and bone structures".* Nationwide Clinical Trials completed in 2008, implemented in clinical practice, Belarus.
- *"Septoplasty of nasal septum with reconstructed cartilage".* Recommended as a standard in Nationwide Clinical Practice starting from 2007, implemented in clinical practice.
- *"Allotransplantation of parathyroid glands to treat severe forms of hypoparathyreosis".* Recommended to and implemented in clinical practice starting from 2001.

**Major Honors And Awards**

- **Erasmus Mundus WEBB Fellowship** The only recipient in medicine. International selection was conducted by European Union in all fields of sciences and humanities.
- **National Prize (First place) "Best Innovation Project by Young Researchers**" Country's most prestigious prize for innovations done by researchers under age 35 in all fields of science and engineering. The prize is awarded for *"Developing, business strategy, and implementation in clinical practice of the new biotechnological medical Textile Material for Reconstruction of Bone and Cartilage Structures"*
- **Second place Award of the President of the Republic of Belarus** Country's most prestigious prize for young researchers. The only recipient in medicine. Nationwide selection was conducted in all fields of sciences and humanities.
- **Second place National Prize for Student Research** The only recipient in medicine. Nationwide selection was conducted by the Ministry of Education in all fields of sciences and humanities.