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Q1
Neural Correlates of Esophageal Speech: An fMRI Pilot Study

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Summary: Objectives. The esophageal speech is one of the possible alaryngeal voices resulting after total laryngectomy. Its production is made by the regurgitation of the air coming from the esophagus, sonorized through the passage from the walls of the upper esophageal sphincter. The neural correlates of this voice have never been investigated, while the neural control of laryngeal voice has been already documented by different studies.

Methods. Four patients using esophageal speech after total laryngectomy and four healthy controls underwent functional magnetic resonance imaging. The fMRI experiment was carried out using a "Block Design Paradigm."

Results. Comparison of the phonation task in the two groups revealed higher brain activities in the cingulate gyrus, the cerebellum and the medulla as well as lower brain activities in the precentral gyrus, the inferior and middle frontal gyrus and the superior temporal gyrus in the laryngectomized group.

Conclusions. The findings in this pilot study provide insight into neural phonation control in laryngectomized patients with esophageal speech. The imaging results demonstrated that in patients with esophageal speech, altered brain activities can be observed. The adaptive changes in the brain following laryngectomy reflect the changes in the body and in the voice modality. In addition, this pilot study establishes that a blocked design fMRI is sensitive enough to define a neural network associated with esophageal voice and lays the foundation for further studies in this field.

Key Words: Esophageal speech–Voice–Neuroplasticity–Larynx–Laryngectomy.

INTRODUCTION

Laryngeal carcinoma is one of the most common malignant neoplasms in the head and neck area, the incidence of laryngeal cancer accounts for about 2.5% of all malignant neoplasms in men and 0.5% in women. In 2017, 150,000 cases were estimated worldwide.[1,2] About 20% of laryngeal tumors can be treated only through total laryngectomy (TL), which remains the golden standard of treatment for advanced infiltrative T4 tumors.[2–4] The intervention of TL consists in the complete removal of the larynx[4] and involves the creation of a permanent opening of the trachea at the cutaneous level in order to ensure a respiratory tract.[5–7] The separation of the airways from the digestive ones leads above all to difficulty of verbal expression.[5–8] To communicate in the absence of the larynx, the laryngectomized subject has to learn a new vocal behavior.

There are several possible ways to restore a "sound emission": artificial laryngeal voice with a voice prosthesis, tracheo-esophageal speech/voice, and esophageal speech/voice.[8,9] The esophageal speech, or the esophageal voice, consists in the elaboration of regurgitation of air coming from the esophagus, sonorized through the passage from the walls of the upper esophageal sphincter. Sound resonance also occurs in the pharynx, in the mouth, and in the nose, with simultaneous articulation using tongue, lips, and teeth.[9,10] The neural correlates of this voice have never been investigated, while the neural control of laryngeal voice has been already documented by different studies. In fact, in a typical situation, the vocal production is mainly attributed to the larynx and from a neural perspective, the larynx sends and receives information mainly from the X cranial nerve, the vagal nerve, and from the spinal nerves (Detailed description in Appendix A).The central control of voice production is a hierarchically organized, related to the different level of complexity and it develops gradually during childhood until control of speech production is achieved. This bottom up neural system goes from the control of innate vocalization (under lower brainstem control) to the control of vocalization initiation, motivation, and expression of voluntary emotional vocalization (under gray periaqueductal (PAG), limbic structures and anterior cingulate cortex control) until the voluntary vocal motor control (under laryngeal motor cortex and its connections)[15,16] (See "Appendix B").

It can be said that the central control of the oral production is conducted by two parallel pathways, the ACC-PAG pathway, responsible for the control of nonverbal and emotional productions, and the laryngeal motor cortex pathway (LMC), which represents the highest level of voice control regulating the motor control of voluntary vocal production, such as speech and song, as well as the voluntary production of innate vocalizations.[12,13,15–18,21–26]

When children grow up, they develop the ability to control innate vocalizations: shrieking can be produced in the absence of pain or suppressed in the presence of pain. The control of innate vocalizations, and thus of emotional states, become voluntary and for this reason, the nuclei of the brainstem and the vocal patterns generator require input from the upper cerebral regions, such as PAG and ACC.[13,15,17,18] PAG received information from ACC and projects to the reticular formation of the lower brain stem. PAG represents an obligatory relay station within the ACC-PAG pathway and plays a gating role in triggering a vocal

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response and modulating its intensity.[12] ACC is involved in the voluntary control of emotional states and that of innate motor patterns, such as vocal initiation and its emotional intonation. The destruction of the ACC causes a loss of voluntary control of the emotional tones during the conversation, akinetic mutism, or even motor aphasia, while the lesions in the PAG lead to mutism. The electrical stimulation of PAG leads to voice production in mammals and in humans, while the stimulation of ACC affects only mammals.[12,13,17,19,20]

The highest level within the hierarchy of the voice control system is represented by the LMC, and its input and output structures. The laryngeal motor area has an extensive cortical and subcortical network (resumed in Appendix C) and is located in the Brodmann 4p area of the ventral primary motor cortex (M1) in the PreCentral Gyrus (PCG) of the frontal lobe. Cytoarchitectonically, other studies reported in a meta-analysis by Simonyan,[11] showed an additional peak of activation in the left premotor cortex (Brodmann area 6), which is similar to the location of LMC in nonhuman primates. Neuroanatomically, the laryngeal cortical motor path dilates in the pyramidal tract, projecting directly bilaterally to the ambiguus nucleus of the brainstem, the site of the laryngeal motor neurons. In fact, due to this bilateral direct projection, the stimulation of LMC in one hemisphere yields to both vocal folds adduction in the larynx, a behavior that is independent of other articulatory movements.[11,16,17,21−23] Although the direct LMC-ambigual connectivity has positive characteristics, like the modulation of brainstem activity, it also includes negative sides such as the inability to control the production of learned vocalization, when the LMC is lesioned bilaterally in humans.[17,24,27] Besides LMC, the so-called laryngeal areas include: superior frontal gyrus (SFG), middle frontal gyrus (MFG) and inferior frontal gyrus (IFG). The frontal gyrus is the seat of the premotor cortex and the supplementary motor area (SMA) which plays a role in the sequential coordination of effector during the vocal production and in motor initiation and planning.[13,17]

The neuroimaging studies on the voice are not very numerous and those on voice disorders are even rarer: currently, the studies in the literature are only related to vocal pathologies, such as spasmodic dysphonia, especially in parkinsonisms, idiopathic unilateral paralysis and muscular tension dysphonia.[14,17,24,29−31] Moreover, almost every previous study has been focused on the impact of neurological voice disorders on the CNS, and only a very limited number of studies investigated the effect of structural dysphonia.[29] Each adaptation in the larynx requires adaptation and updating of neural models for neural plasticity reasons[23]: numerous studies have shown that when one part of the body is deprived, as after amputation of a limb, somatosensory and motor cortices undergo neuroplastic changes leading to phenomena such as a phantom limb.[32] In laryngectomized patients adaptation of the altered anatomy occurs, which probably also requires reorganization of cortical input from the central nervous system. However, these central and adaptive mechanisms have not yet been described.

The present study attempts to address this problem using functional magnetic resonance imaging (fMRI). The main objective is, therefore, to study the brain activity during phonation in laryngectomized patients with an erigmofonic voice compared to healthy controls, focusing in particular on the activation of the laryngeal motor control areas (LMC, PCG, SFG, MFG, IFG), investigating plasticity mechanisms. The first (1) hypothesis is to find altered brain activity related to voice control, due to the absence of larynx. In particular, we expect a lower activation of laryngeal areas in laryngectomized subjects. (2) A secondary objective is to evaluate the activation of other brain areas such as the medulla oblongata in laryngectomized patients with an erigmofonic voice. In the patient with an erigmophonic voice, the subject vocalizes by performing specific eruptions, taking air from the stomach and passing it through the esophagus and the pharynx; for this reason, the third (3) hypothesis is that, in the absence of larynx and vocal function of the lungs, there could be a greater activation of the postrema area, located in the medulla oblongata, which is responsible for the eruption in healthy subjects.[33]

MATERIAL AND METHODS

From August 2018 to September 2018, 10 subjects who had undergone TL and using esophageal voices were recruited.

The inclusion criteria adopted were:

1. Subjects whose signature of informed consent has been collected (also by a neighboring family member)

For the larynctomizedgroup in particular:

1. Having been subjected to a TL.
2. Use of the esophageal speech.

For the control group:

1. The age has to be matched with the case-group.

The exclusion criteria adopted are:

1. Neurological disorders
2. Diagnosis of dementia, or other cognitive impairment
3. Pregnancy
4. Claustrophobia
5. Pacemaker/cardioversion defibrillator
6. Metal clips, that is metal, noncompatible biomedical devices

Clinical-anamnestic data was anonymised and pseudonymised, collected in an excel database and only the study participants had access to it.

The protocol of the study has been accepted by the Ethical Commision of the Hospital “Maggiore della Carità” of Novara, Italy.

Sample recruitment

The total laryngectomized subjects have been contacted via the Italian AILAR-laryngectomized association. The study project was presented during an esophageal voice lesson at the headquarters of the AILAR association of Novara. Subsequently, the subjects who decided to join the project were contacted personally by telephone to check the inclusion and exclusion criteria.

For the control group, the subjects recruited were age-matched patients. These were already supposed to undergo morphological MRI for cephalgia and they were asked to perform fMRI for generic experimental reasons. They have been kept blinded and signed a dedicated informed consent.

Tasks

Before the starting of the fMRI protocol, each subject met the speech-language therapist of the Department of Otolaryngology of Novara for training in the production of different tasks in a natural way, trying to minimize orofacial movements. An informed consent form, an information module and the magnetic resonance questionnaire (in the Italian language) were delivered before the fMRI acquisition and signed under the Radiologist supervision.

During the study protocol conducted at the Department of Radiology, the cortical activity of the subjects has been evaluated through the fMRI, subjecting each subject examined to vocal tests.

The protocol was inspired by different studies: a study by Krishtopava and colleagues about phonation in function muscle dystonia,[17] and a study on neural bases of primary muscle tension dysphonia from Nelson Roy.[31]

The functional magnetic resonance protocol provides one unique task to perform in one session of scanners completed on the same day. The activity has been presented in a block design in which six 30-seconds rest blocks and six 30-seconds active blocks alternated for a total of 6 minutes and 30 seconds per activity starting with the rest period. During active blocks the patient has been instructed to repeat in comfortable tones a sustained vowel "i" for about 5 seconds with an interval of 5 seconds between a vowel and the next, resulting in 3 productions during the active block of 30 seconds.

The production of the sound /i/ was chosen because this sound requires a slight labial and dental opening, the positioning of the tongue at the bottom in an anterior position. Compared to other sounds, the movement of the orofacial muscles is minimal. The periods during which the subject produced the different tasks have been announced through the MR-compatible headphone.

The data obtained have been stored anonymously in a virtual database with access restricted to studio employees only.

Acquisition of functional magnetic resonance data

Magnetic resonance images were acquired with a Philips Ingenia 3- Tesla magnetic resonance imaging (MRI) at the Radiodiagnostic Department of the Hospital of Novara. The subject's head was firmly secured using a standard head coil and ‘‘memory’’ pillow. Earplugs were used to help block out scanner noise and to give commands. Subjects performed each task as 30 epochs of an oral task alternating with 30-seconds epochs of rest. During the protocol, the instructions were communicated via earphones. The alternation of the activity phases and the rest phases and the beginning and the end of the production of the task in the active phase have been signaled via headphones. All stimuli were created and presented using NordicAktiva Software.

The functional magnetic resonance data were obtained with a sequence T2 *—eco planar weight (EPI) (TR = 3000; TE = 35; flip angle = 90°; FOV = 230; matrix dimension 94×94). 30 slices were acquired in interleaved mode with a thickness of 4 mm each with a spacing of 1 mm between one slice and the other and orientation of 30° in a clockwise direction with respect to the plane passing through the front and back joints (AC-PC); this to prevent the artifact from susceptibility

For each subject, the total number of 126/126 of volumes are acquired for a scan time of 18 minutes.

Image analysis

The images from fMRI were processed off-line to generate statistical maps of brain activity using IViewBold on proprietary workstations (Intellispace, Philips, Belgium) with clusters and thresholds set both at 3 Preliminarily in all subjects, the functional magnetic resonance images were motion-corrected in 6 directions by realignment of the set of images to the first image in the subtracted set. Motion correction is necessary for fMRI studies to eliminate in-plane or cross-plane head motion, which produces signal intensity artifacts that appear identical to brain activity. Imaging with head movement on the 3 axes over the 3 mm was excluded from the analysis. The time-series data were convolved with a hemodynamic response function incorporating the hemodynamic delay. Therefore, the onset of activity resulting from local hemodynamics lags the neural activity by 6 seconds. Corrections for local and regional variations in signal and filtering of noise (eg, cardiac- and respiratory induced noise) were performed by spatially smoothing the data with a Gaussian smoothing kernel of 8 pixels at full width half maximum and applying high- and low-pass filters.The determination of BOLD signal activation maps in patients after TL and in healthy controls was obtained following a block paradigm, comparing the rest periods with the active phases within each subject.Then, both images structural and functional ones have been coregistered. The statistical maps deriving from the first-level analysis have been visualized applying a threshold per cluster with a threshold Z equal to 3 and a threshold of significance of the cluster of $t < 0.001$. Subsequently, an analysis was carried out between groups to search for any differences in BOLD activation between patients with esophageal speech and healthy controls; therefore, it was conducted statistical analysis with t test for unpaired data.

RESULTS

From the 10 subjects, 6 have been excluded: 4 subjects for health reasons and 2 because of exclusion criteria, such as the presence of metallic clips.

In addition, 4 controls of comparable age were examined and selected among subjects who were supposed to submit the fMRI for other reasons not related to our study.

A total of 4 laryngectomized subjects and 4 healthy subjects underwent functional imaging in this investigation. The group of patients has a mean age of 66,25 years old and contains 100% of males.The control group, on the other hand, has an average age of years of 65,5 years old and is also made up by males only. In the case groups, all the participants underwent TL at least 2 years ago and they use an esophageal speech from at least 1 year. More precisely 2 subjects have used it for 6 years, one subject for 2 years and one for 1 year. This data reflects the different abilities of the participants in performing the task. The demographic and clinical data of the participants are summarized in Tables 1 and 2. The group of patients with esophageal voice and the control group do not show statistically significant differences in age and sex. Within the group of controls, they do not differ by age. Within the group of patients, they do not differ significantly by age but that is a significant difference in time of acquisition of the voice after the intervention. This last data reflects the different skill levels of the subjects in the use of the esophageal voice.

Behavioral analysis

All subjects reported comfortable use of the device. The average absolute displacement of the head during acquisition functional images were less than 3 mm for both patients and controls.

All subjects from the control group have performed the task correctly.

In the case group, only one subject performs the task without any modifications. This subject is an esophageal speech teacher, and therefore he can use and modulate the voice in a perfect manner. Other two participants emitted a shorter /i/ in the onset phase. One patient was suffering of chronic obstructive pulmonary disease and therefore he couldn't perform the all task in the laying position, but only in the standing position, thus he attempted to perform the task as best as he could. This results in an over activation of different areas, with several artifacts that were too high to differentiate them from the areas of interest. One patient with esophageal speech was therefore excluded from subsequent analyses due to overactivation of these areas, but it is worth analyzing this in the context of further studies.

TABLE 1.
Demographic Datas of Case and Control Group

	Number	Age	Sex
Patients	4	66,25	4 M
Controls	4	65,5	4 M

TABLE 2.
Clinical Datas of Patients

	TL	Time From TL	Use of ES
S1	2016	4	1
S2	2012	6	6
S3	2011	7	2
S4	2012	6	6

fMRI analysis

Within-group analysis

From the comparison between active periods and rest periods in the group of patients with esophageal speech, a significant bilateral activation with a left dominance emerged at the level of the STG corresponding with the left 34,38,41,42,43 Brodmann areas (BA) and with the right 41,42,43 BA and at the level of the PCG (4 BA). Significant activation of the cingulate gyrus (26,29,30,31 BA) and of the medulla was detected. Hyperactivation of the cerebellum with higher activation of the right side with a peak of activation in the 3 and 6,7 lobuli, and a lower action in the left side with the 4 lobulus.

The results are summarized in Table 3.

In the control group, the areas that were significantly active at comparison between active periods and rest periods, are represented by the bilateral precentral gyri, with higher activation in the left hemisphere (3,4 BA) and lower in the right one (4 BA), and by the inferior and middle frontal cortex, these areas are included in areas 9 and 46 of Brodmann. The cingulate cortex is significative activated, and STG is also hyperactive bilaterally with a left

TABLE 3.
Brain Activation During Phonation in Laryngectomized Subjects

Phonation in the Laryngectomized Group			
Area	Brodmann Area	T peak	Dimension (mm^3)
Right precentral gyrus	4	3,6	46
Left precentral gyrus	4	3,9	50
Right superior temporal gyrus	41,42,43	3,4	269
Left superior temporal gyrus	38,41,42,43	4,1	740
Cingulate gyrus	26,29,30,31	4,1	1561
Medulla oblongata		3,1	496
Right cerebellum		3,9	293
Left cerebellum		4	4279

TABLE 4.
Brain Activation During Phonation in the Healthy Group

Phonation			
Area	Brodmann Area	T Peak	Dimension (mm^3)
Right precentral gyrus	4,6	4,1	400
Left precentral gyrus	4,3	4,6	1350
Right middle frontal gyrus	46	3,5	120
Left inferior frontal gyrus	9	4,1	120
Cingulate gyrus	31	4	780
Right superior temporal gyrus	41/42/43/38	3,8	588
Left superior temporal gyrus	41/42/43	4,1	564
Right cerebellum	3,6,7	3,6	434
Left cerebellum	4	4,1	88

dominance. Significant cerebellar activation is present in both sides and in this case the activation is greater in the right cerebellum. The results are summarized in Table 4.

Between-group analysis

Subsequently, a comparative evaluation was carried out between the case group and the control group. There was a significant increase in the BOLD response in the control group in the right STG, in the right middle frontal gyrus and, particularly, in the left precentral gyrus (medium volume 1660). The motor laryngeal cortex, corresponding with 4p BA, showed an activation peak ($P < 0.001$, cluster Z = 3) in all healthy subjects, unlike the laryngectomized group. On the contrary, in the BOLD sequences at the subtentorial level, the medulla was found to be hyperactivated exclusively in the group of laryngectomized patients (Figure 1). The cingulate gyrus and the cerebellum have been found to be more active overall in the laryngectomized group with an hyperactivation of the areas. The activation of the cingulate was also more distributed in the laryngectomized group than in the control group. The results are summarized in Tables 5 and 6.

DISCUSSION

The purpose of this study was to investigate cortical and subcortical activation changes after TL and therefore plasticity mechanisms and to evaluate the activation of other brain areas, such as the medulla oblongata. We have hypothesized about different brain activation in laryngectomized subjects using esophageal speech, particularly, in the laryngeal motor control areas (LMC, SFG, MFG, IFG, insula, midbrain) and about a greater activation of the medulla oblongata, which is responsible for the eructation in healthy subjects.

Four men after TL with esophageal speech and 4 healthy men participated in the study. The paradigm explored the neural control associated with phonation.

In the study, the brain activity in response to phonation of /i/ shows bilateral activation of the precentral gyrus, the superior temporal gyri, the cerebellum, the cingulate gyrus and unilateral activation of the inferior and middle frontal gyri in healthy subjects. These findings are in line with recent fMRI studies including simple voice production tasks[17,21–23] (Appendix D).The areas observed in the previously reviewed studies are specialized for different functions: the precentral gyrus and the middle and inferior gyri have been, with the proper laryngeal areas, deemed responsible for voice production due to vocal folds movements in different studies by Kryshtopava.[28] These areas, which include M1 and SMA are involved in the initiation and execution of voluntary vocalization. Brown has identified the VI lobule of cerebellum as also being primary areas of motor laryngeal control.[22] The STG and the MTG control auditory feedback and self-monitoring. The cingulate cortex activity is associated with the initiation of control and emotional modulation, while the cerebellum is involved in motor planning and coordination.[17,24] In the laryngectomized-subjects group, the main activations were found in the PCG, the STG, the cingulate cortex, the cerebellum, and the medulla oblongata. Compared to the healthy group, greater activation of the cingulate cortex, left cerebellum and medulla oblongata was observed, while the activation of the inferior and middle frontal cortex, the left STG and of the PCG was lower compared to the healthy group.

The imaging results supported the hypothesis that subjects with esophageal speech may have altered brain activity related to phonation control.

We hypothesized that the laryngeal areas would have shown a lower activation, while different areas, like the medulla oblongata, would have shown higher activation in the laryngectomized subjects using esophageal speech.

The laryngeal areas, including PCG, IFG, and MFG, allow voluntary modulations of pitch, intensity and harmonious quality of vocal production in healthy subjects. Especially the IFG and the MFG, seat of the SMA are involved in the control of more complex voice production, such as syllable or speech. While MFG and IFG activation was not significant, the PCG showed significant activation in this group. Those study findings show that these areas are more involved in the laryngeal voice, which is a more refined voice, compared to the esophageal voice, which is rougher and more transpiring with a low tone and a reduced volume and which is related to the automatism of eructation. Nevertheless, even if with a lower activation, the PCG has been involved in the production of alaryngeal voice during our protocol. There are different explications why this area may be activated even in the absence of the larynx. First of all, even if the target of the paradigm was to detect neural correlates of voice production while performing a /i/, the

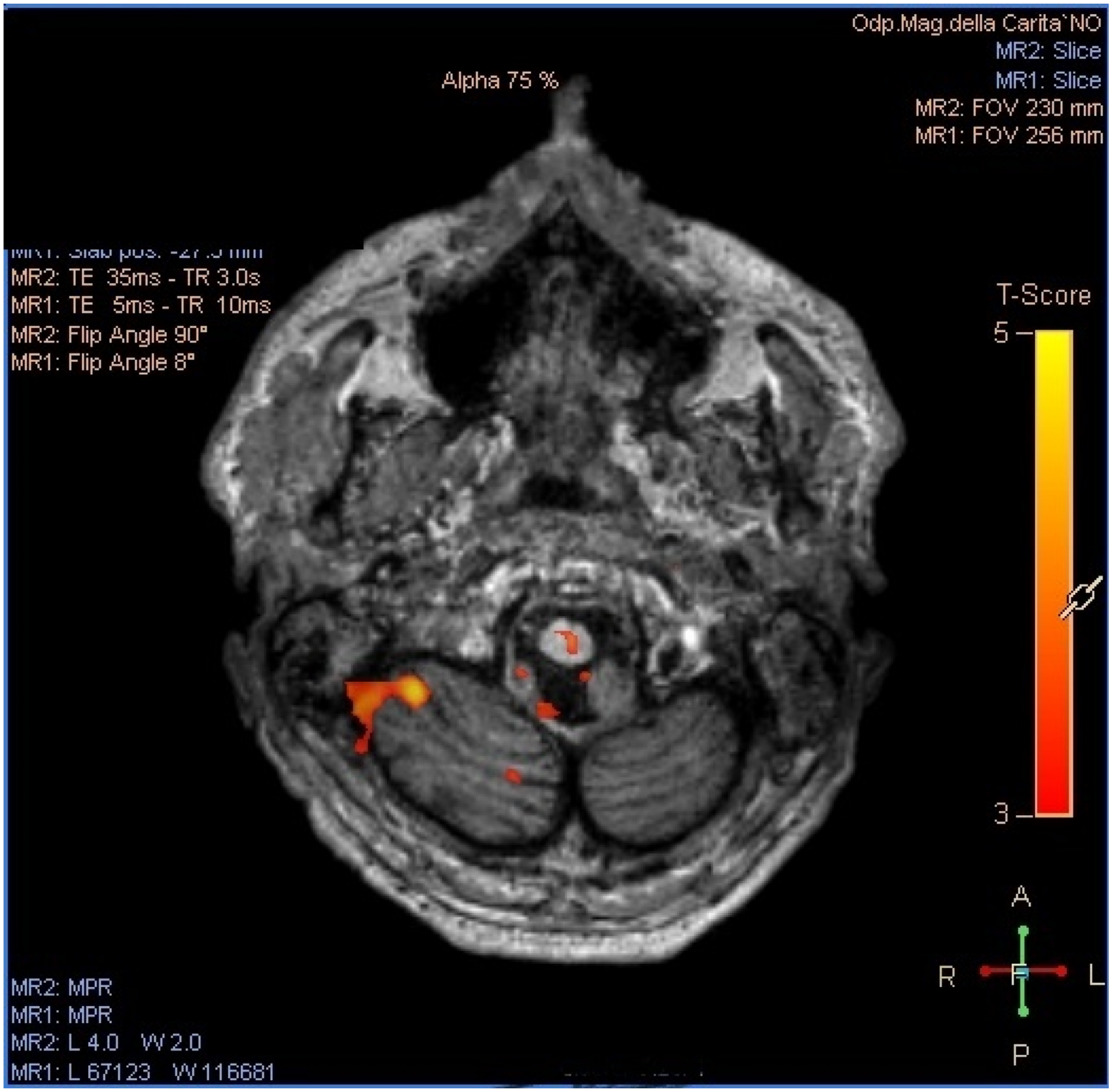

FIGURE 1. Activation of the medulla and of the right cerebellum in laryngectomized subjects while performing the /i/ task ($P < 0.001$, cluster Z = 3).

TABLE 5.
Areas With Higher Activation in Control Group Compared With Laryngectomized Group

Healthy Group > Laryngectomized Group						
	Healthy Group			Laryngectomized Group		
Area	BA	Cluster (mm^3)	T value	BA	Cluster (mm^3)	T value
Right PCG	4,6	400	4,1	4,6	46	3,6
Left PCG	4,3	1350	4,6	3,4	50	3,9
Right MFG	46	120	3,5	-	-	-
Left IFG	9	120	4,1	-	-	-
Right STG	41/42/ 43/38	588	3,8	41,42, 43	269	3,4

TABLE 6.
Areas With Higher Activation in Control Group Compared With Laryngectomized Group

Laryngectomized Group > Healthy Group						
	Healthy Group			Laryngectomized Group		
Area	BA	Cluster (mm3)	T value	BA	Cluster (mm3)	T value
Cingulate cortex	26,29, 39,31	1561	4,1	31	780	4
Medulla oblongata		4279	4	-	-	-
Left cerebellum		4279	4	4	88	4,1

production of this vowel, although requiring less orofacial movements than other phonemes, involves in minimal part the movement of the tongue, jaw, and lips. Moreover, in the esophageal speech, in order to emit a sound, the tongue injects air into the esophagus and therefore it has to move even for producing the /i/ vowel. These considerations lead to the first assumption, according to which an overlapping activation between orofacial regions in M1 and the LMC could exist. This has been reported in different studies resumed in Appendix E. Even hypothesizing that the proposed task does not involve other movements except those laryngeal and that the localization of the larynx within the motor cortex is specifically the one observed also in laryngectomized subjects, namely the Brodmann area 4, other explanations can still be taken in account.[14,16,17,21,22,25,26] In the brain of subjects missing the larynx, a plasticity phenomenon, a reorganization of the brain itself when the sensory input is lost, can occur, but the original functions of a given brain area are not deleted. Scientists had so far focused more on the study of the changes of brain images of the amputee's remaining limbs, to trace possible modifications. In a recent ultra-high-field neuroimaging study by Kikkert and colleagues, individual fingers persist in the primary somatosensory cortex even decades after arm amputation. The finding reopens the question of what happens to a cortical territory once its main input is lost and how long a continued sensory input is necessary to maintain organization in sensorimotor cortex.[39] Also in our study, the brain activity of the laryngectomized was weaker compared to the control group, but not absent. Moreover, a joint action of the laryngeal area and the cingulate area may control the volitional nature of vocal production.

The cingulate area

The cingulate area, compared to the control group, shows a higher and more distributed activation, with the involvement of the posterior and middle cingulate gyrus and of the anterior cingulate cortex as well.

This area is associated with voluntary motor control for phonation, especially during emotional vocal modulations. The cingulate complex is involved in the voluntary control of emotional states of innate motor patterns, such as vocal initiation and its emotional intonation. Moreover, the middle cingulate cortex is involved in conflict monitoring, willed action, and action selection. The cingulate cortex and gyrus as well, take place, with the PAG, in the second level of the hierarchical organization model proposed in different studies and already explained in the second chapter. The automatic vocalization, such as crying, laughter or scream, is controlled and modulated by the subcortical regions of the PAG and the cingulate gyri. Thus, the initiation and the emotional control of esophageal speech production can be controlled by these areas too. In fact, the esophageal speech is made by the automatism of eructation.

The hyperactivation of the cingulate gyri could also reflect the strong will that these subjects invest in producing a new voice and the importance of the emotional control for the production of this voice. In fact, the cingulate area is involved in emotional voluntary control and, when it is lesioned, leads to a state in which the patient has a defection of emotional intonation and initiation.[40]

Medulla oblongata

Significant activation of the medulla has been detected in the patients group, compared to the control group: this activation corroborates with the hypothesis of the study and is not shown in healthy subjects. The medulla is part of the brainstem, a region of the brain which is responsible for reflex and innate actions, such as swallowing or eructation. This finding can be explained with the physiology and neurophysiology of eructation studied by Lang in 2015.[33] In fact, the esophageal speech, produced by laryngectomized subjects, consists of the elaboration of an eructation of air coming from the esophagus, sonorized through the passage from the walls of the upper esophageal sphincter. The physiologic eructation is composed by three independent phases: gas escape through the lower esophageal sphincter relaxation, upper barrier elimination through the upper esophageal sphincter relaxation and gas transport phases. It is a rapid movement and involves complex motor event of different body systems, that is, the digestive tract, the respiratory systems, and the orofacial systems. In esophageal speech, there's no involvement of the lungs or of the stomach, only of the esophageal tract and the oral cavity. The esophageal voice includes only the second phase, because the air is injected from the mouth and doesn't have to reach the stomach. Studies in cats have found that by stimulating the esophageal receptors with air pulse injection, the upper barrier elimination phase, the area postrema (AP), subnuclei of the nuclei tractus solitarius and the nucleus ambiguus are activated.[33] One unique aspect of neuronal activation during this phase is the very significant activation of localized areas of the AP, situated in the medulla oblongata. In fact, It has been hypothesized, based on numerous studies on the neural control of AP on emesis, that the AP is the major integration nucleus also for activation of eructation.[33] Moreover, the upper part of esophagus is made by striated muscles, its contraction is initiated and generated by swallowing pattern generator (SPG) located in the brainstem.[41] Thus, the higher activation shown in laryngectomized subjects can be due to this assumption. As a voice made by the production of the eructation, its neural control may be controlled by the lowest level of voice control, that is the brainstem. However, the esophageal voice represents a finer and more structured action than mere belching. For this reason, the voluntary production of the esophageal voice may require the intervention of higher brain areas such as the activation of the cingulate gyrus and of the motor area which have a joint role in the voluntary production of voice as mentioned before.

Cerebellum

Increased degrees of activation were also shown in the cerebellum. The cerebellum is known to have a role in plasticity[42] and to have projections to motor regions in the frontal lobe, creating a cerebellar feedback loop for muscle movements. Activation in frontal premotor and primary motor areas and the cerebellum may demonstrate the brain's skill in adapting to changes in motor performance.[29] Furthermore, the coordination role of the cerebellum has been studied in depth and the higher activation of this area may reflect a major need of coordination of the new structures involved in the esophageal voice, which are different from the previous ones used for the laryngeal voice produced before TL.

Superior temporal gyrus

Besides the greater activation of the laryngeal areas, the fMRI analysis uncovered increased activity in the left STG in the control group. The STG has been associated with auditory feedback and self-monitoring of voice production.[24,28] This hyperactivation in the subjects may be explained by acoustical reasons. In fact, the laryngeal voice compared to the esophageal speech, presents a higher tone and volume which may result in higher auditory feedback. Moreover, the loud sound of the fMRI may cover the already low-volume esophageal speech and thus can interfere with the auditory feedback and self-monitoring role of the STG in laryngectomized subjects. On the contrary this result may be in controversy with results of an fMRI experiment conducted by Parkinson and colleagues,[43] who have investigated the neural activations related to audio vocal responses using a pitch-shift perturbation paradigm. In this study, the STG activation was higher during pitch-shifted compared with nonshifted vocalization. It had been suggested that a match between expected and actual output results in suppression and overlapping pattern of activations in the auditory cortex, while a mismatch between expected and actual output results in an increase of sensitivity in the temporal gyrus. The esophageal voice has different vocal characteristics compared to the laryngeal voice that these subjects used to have. The sound is reduced in volume and in tone, thus a mismatch between the output that has always been expected and the actual output results could have been reflected in overactivation of the STG. This aspect needs further studies.

Limitations of study and recommendations for future research

Although the data presented here provide evidence for the altered brain activity related to voice control in laryngectomized subjects, this study has several limitations. As a pilot study, the patient sample size is small and a larger sample sizes may be needed to confirm whether this finding represents subject variability. Moreover, there is no detailed classification of the patient group and this was an important aspect to consider in further research: in fact, only one subject managed to perform the task without any modification. This subject was an esophageal speech teacher, and therefore he could use and modulate the voice in a perfect manner. The other two participants emitted a shorter /i/ in the onset phase. This inability to pronounce a long sound was probably due to the lying position, for which it was more complicate to inject aria into the esophagus e regurgitate it. This ability was related to the time of esophageal speech use: the teacher has used the voice for 6 years while the other two subjects for less than 2 years. Noteworthy, an higher activation of medulla and a lower activation of laryngeal areas was identified in this patient: this may reflect the occurrence of more complete plasticity mechanism.

The fact that laryngectomized patients were subjected to chemotherapy could have represented another limitation: a study of Saykin and colleagues reports changes in cognitive functions following chemotherapy which may affect fMRI results.[44]

The analysis of the paradigm was performed using IView-Bold on Intellispace Philips and only compared with SPM software. In the continuum of the experiment a second level analysis will be needed to confirm or modify the results.

Additionally, another limitation of the study is that although the tasks were the same for case and control group in a general sense, the type of voices was different, laryngeal and alaryngeal voices requiring different body structures. A golden standard would have been to compare the esophageal voice in laryngectomized subjects and healthy subjects or a subject before and after TL. We have considered the healthy group as the group before TL, and, therefore with a larynx. We have considered doing proceed like mentioned above, but the esophageal speech is difficult to learn while having the larynx. The research hypothesis for future studies is to perform a longitudinal study on subjects pre and post TL, in order to see the changes within the same subjects.

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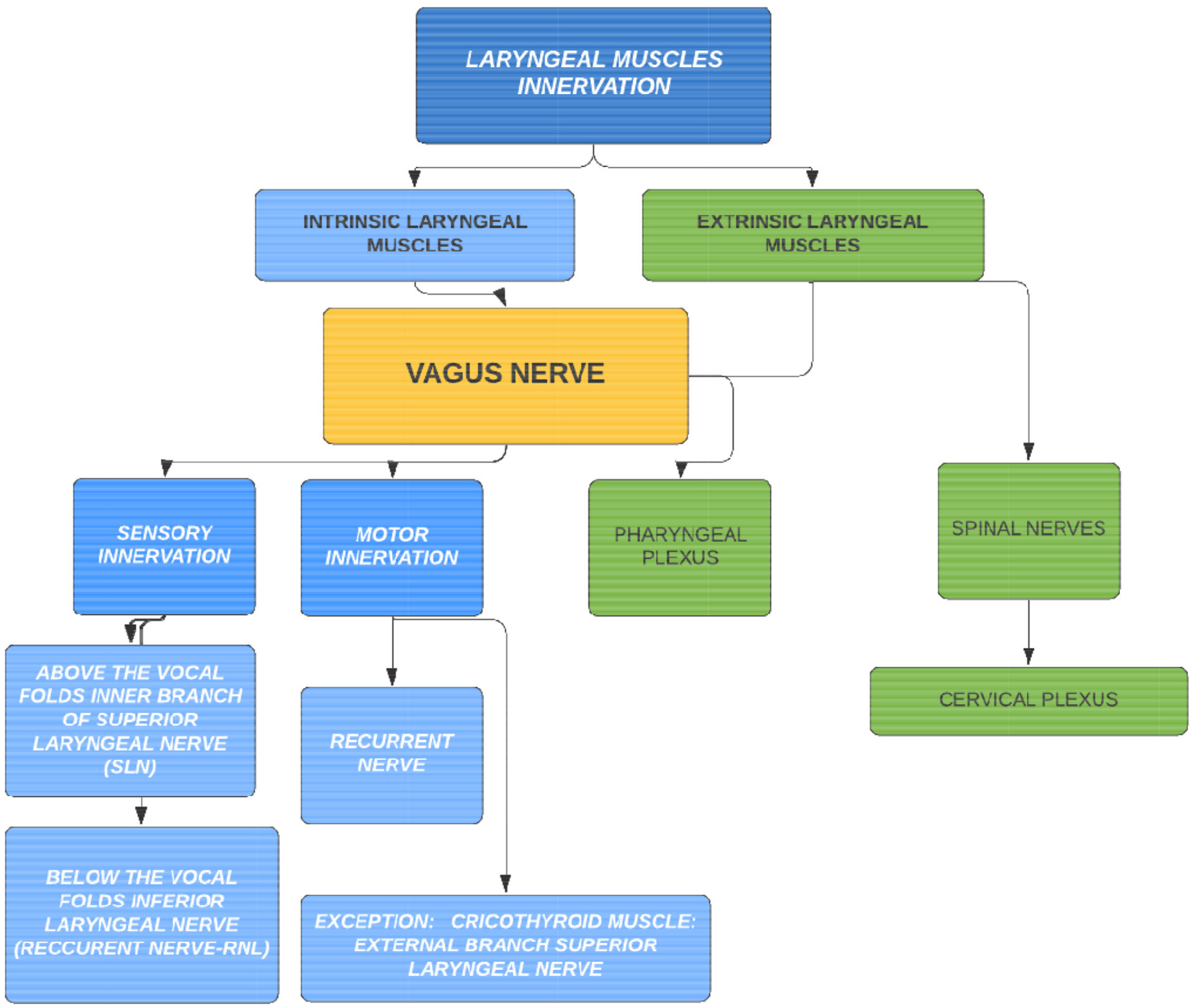
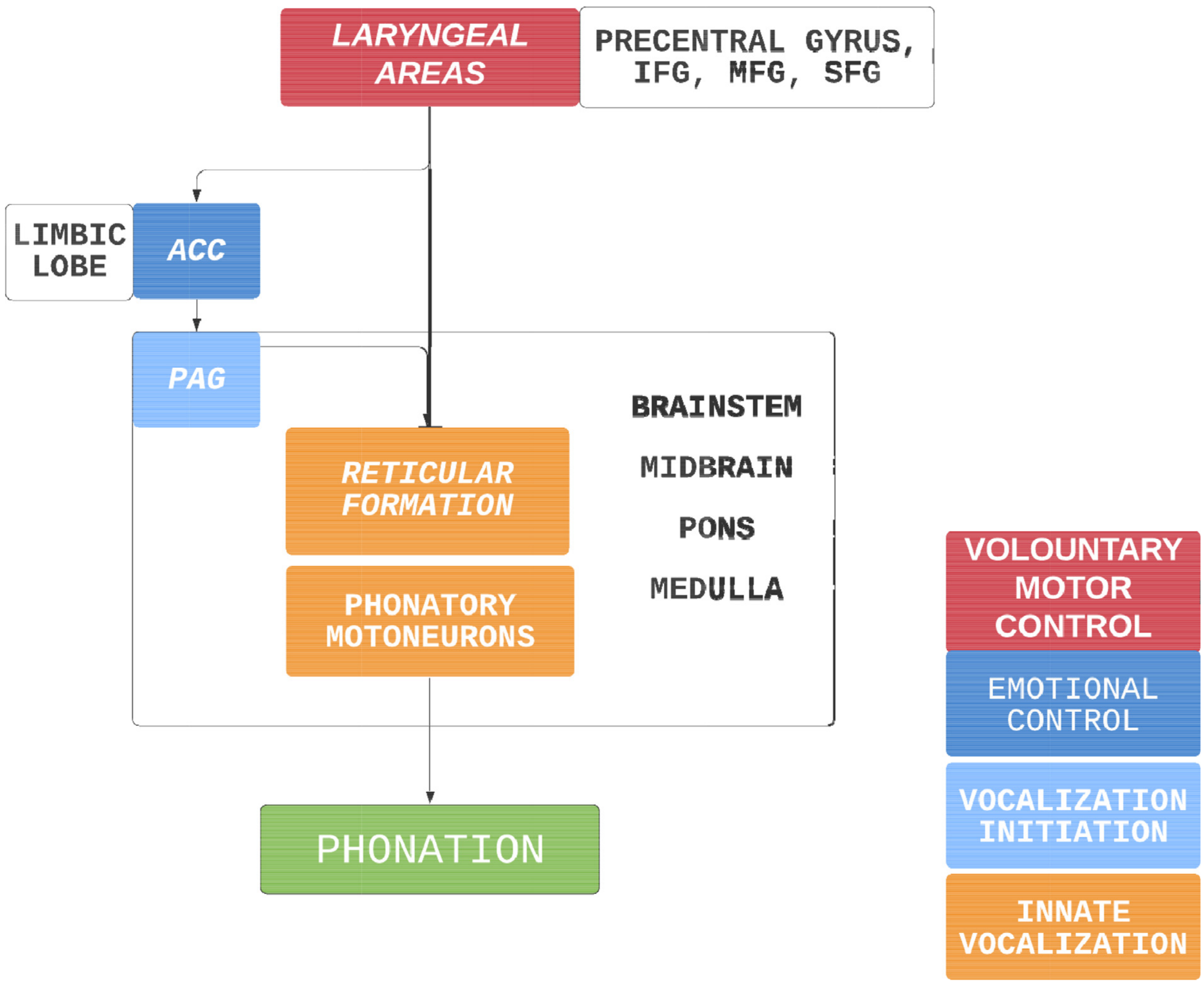
APPENDIX A



The vocal production is mainly attributed to the larynx and from a neural perspective, the larynx sends and receives information mainly from the X cranial nerve, the vagal nerve, and from the spinal nerves. The intrinsic laryngeal muscles receive bilateral motor and sensory innervation from branches of the vagus nerve: the inner branch of the superior laryngeal nerve (SLN) and of the inferior laryngeal nerve, also called recurrent nerve (RLN), ensures sensory innervation, while the external branch of the recurrent nerve (RLN) guarantees motor innervation. The cricothyroid muscle (responsible for lengthening of the vocal cords) is an exception, being innervated by the external motor branch of the SLN.[11–13] The extrinsic muscles of the larynx are innervated by the cervical plexus (spinal nerves) or the pharyngeal plexus.[14,15]

APPENDIX B



The hierarchical organization is related to the different level of complexity in voice production. The screech present at the birth of the newborn, the crying, the laugh of a child, represent the lowest level of vocal control: these basic innate vocalizations are completely genetically determined vocal reactions, which means that an infant doesn't need to hear these sounds previously to reproduce them.[12,13] Their production is due to brainstem activation. The nuclei of the ambiguous solitary tract, trigeminal, facial, hypoglossal, solitary and of the spinal cord, thoracic and lumbar ventral horn, are responsible for the coordination of basic muscular laryngeal, respiratory and articulatory activity.[13,15,17] The connection of the different nuclei is assigned to the reticular formation (RF) of the pons and of the lower brain stem. The RF plays a crucial role in vocal motor coordination and represents the basic level of the execution of this path.[15] When children grow up, they develop the ability to control innate vocalizations: shrieking can be produced in the absence of pain or suppressed in the presence of pain. The control of innate vocalizations, and thus of emotional states, become voluntary and for this reason, the nuclei of the brainstem and the vocal patterns generator require input from the upper cerebral regions, such as PAG and ACC.[13,15,17,18] PAG received information from ACC and projects to the reticular formation of the lower brain stem. PAG represents an obligatory relay station within the ACC-PAG pathway and plays a gating role in triggering a vocal response and modulating its intensity.[12] ACC is involved in the voluntary control of emotional states and that of innate motor patterns, such as vocal initiation and its emotional intonation. The destruction of the ACC causes a loss of voluntary control of the emotional tones during the conversation, akinetic mutism, or even motor aphasia, while the lesions in the PAG lead to mutism. The electrical stimulation of PAG leads to voice production in mammals and in humans, while the stimulation of ACC affects only mammals.[12,13,17,19,20]

APPENDIX C

Areas	Function
Superior frontal gyrus (Supplementary motor area—SMA)	Plays a role in the sequential coordination of effector during the vocal production and in motor initiation and planning.[13,17]
Middle frontal gyrus	Vocal self-monitoring and processing[12,13,17]
Inferior frontal gyrus	Responsible for motor planning, in particular, its pars opercularis is responsible for hierarchical sequencing of linguistic and nonlinguistic sequences. It is active during production of long sequences, while its activation during production of single syllables is less probable.[12,22]
Superior temporal gyrus (STG)	Sensory guidance of movement and regulates the larynx before reaching the phonatory task. It receives auditory feedback through the RF from the ascending sensory auditory pathway and it is responsible for speech processing[12,13]
Middle temporal gyrus (MTG)	Self-voice control.[12,13]
Parietal gyrus- inferior and superior (IPG-SPG)	These areas are involved in multimodal sensory integration and perpetual calibration[29]
Cerebellum	Plays a role in the sequential coordination of effector during the vocal production[12,13,17]
Antero-lateral parietal lobe (somatosensory cortex- post-central gyrus)	It is important for the integration of proprioceptive feedback from oropharyngeal, respiratory and laryngeal regions during voice production. The somatosensory cortex modulates the orofacial articulators for vocal motor control[12]
Thalamus	With the parietal lobe, thalamus is associated with central monitoring and modulation.[12,13,17]
Insula	With parietal lobe, it integrates sounds with the emotions and attitudes of the speaker.[17]

APPENDIX D

	Activation during phonation
Kryshtopava et al[34]	Precentral gyrus, the SFG, the posterior cingulate gyrus, the STG, the MTG, the insula, and the cerebellum, the left MFG, the IFG, and inferior parietal lobe, the cingulate gyrus, lingual gyrus, and the thalamus
Vigneau et al[35]	Primary motor, somatosensory, and auditory cortical areas, the medial and lateral premotor areas, the inferior frontal gyrus, the superior temporal gyrus, the anterior insula, the subcortical regions including the medial and lateral cerebellum, the basal ganglia, and the thalamus
Indefrey and Levelt[36]	Primary motor, somatosensory, and auditory cortical areas, the medial and lateral premotor areas, the inferior frontal gyrus, the superior temporal gyrus, the anterior insula, the subcortical regions including the medial and lateral cerebellum, the basal ganglia, and the thalamus
Loucks et al[21]	A study by Loucks and colleagues has shown activation during phonation in the lateral sensory, motor, and premotor regions of the left hemisphere, the bilateral dorsolateral sensorimotor regions, the right temporoparietal, cerebellar and thalamic regions, the supplementary motor area and the anterior cingulate cortex

Studies investigating the neural activity during phonation, which corroborate with our study.

APPENDIX E

Miyamoto et al[26]	Dorsoventral somatotopic organization for the lips, jaw, tongue, and pharynx, respectively
Jürgens[27]	Association with the brodmann area 4p with the whole facial area, responsible for both lips, jaw, tongue and vocal fold movements
Brown et al[22]	Also, Brown and colleagues attempted to disentangle articulation and phonation areas in a meta-analysis in 2009. This analysis confirmed a strong overlap in the larynx motor area, with the only differences in brain activity being in the rolandic operculum, more connected with the tongue movements.
Terumitsu et al[37]	Topographically dorso-ventral ordered positions within the primary sensorimotor cortex for lip and tongue movements, while the location of a LMC in the primary motor cortex appears less clear with two recent studies reporting different positions for laryngeal area among lips or tongue motor areas.
Grabski et al[38]	Grabski's research group confirmed that vowel vocalization and nonspeech orofacial movements have been shown to involve very similar activation of the sensorimotor system, besides specific auditory and phonological activations in the bilateral temporal cortices for vowel vocalization. They have identified a functional motor network controlling both laryngeal and supralaryngeal movements, which include the sensorimotor and premotor cortices and bilaterally, the right inferior frontal gyrus, the supplementary motor area, the left parietal operculum, and the adjacent inferior parietal lobule, the basal ganglia, and the cerebellum.

Articles investigating the overlapping activation between orofacial regions in M1 and the LMC.