UNIVERSITÀ DEGLI STUDI DI MILANO

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The neural network underlying speech in humans: intraoperative investigation of motor control of speech in Broca, ventral pre-motor and primary motor cortices

BIO-09

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Ad Enzo

“E’ assai noto
che non c'è uomo tanto ebete e stupido, neppure un pazzo,
che non sia capace di mettere insieme diverse parole
e farne un discorso per comunicare il suo pensiero;
e che al contrario
non c'è altro animale, per quanto perfetto e felicemente creato,
che possa fare lo stesso.”

Discorso sul metodo, parte V, 1560
René Descartes

“Una ricerca biologica sul linguaggio appare necessariamente paradossale
dal momento che viene così ampiamente ammesso
che le lingue consistono di convenzioni culturali di natura arbitraria.”

I fondamenti biologici del linguaggio, 1967
Eric Lenneberg

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1. *Introduction*

Language is a unique and essential instrument of human thought and communication, so much so that Charles Darwin argued that "language is an instinct, exactly like the upright posture" (Moro et al., 2003). It consists of lexical items expressed following phonological, semantic and syntactic rules, and, when it is used in verbal communication, these internal representations are translated in sounds by means of sensory-motor system able to connect internal word to the external one.

The language communication can be subdivided in verbal communication (or speech production), listening and reading. In speech production, human activates a lexical concept and selects the corresponded-lemma (words). After that, human selects in sequential way the sounds (or phones/phonemes: distinct language sounds) composing the target words's morphemes (a morpheme is the smallest meaningful unit, not identical to a word) and these spelled-out segments are successively clustered in syllabic patterns. As syllables are incrementally created, they are rapidly turned into motor action instructions. The produced-words are combined in proper hierarchical structures to form a sentence, by means syntactic rules, while the semantic rules control the meaning of the whole sentence, on the basis of the meaning of each lexical item. In listen and read words/sentences, instead, human codes the phonemes and the graphemes, respectively, and retrievals and selects the corresponded-lemma.

Before to begin this treatise, a first important distinction must be made between two terms apparently synonyms: *language* and *speech*. The term *language* underlies the complex ability to use a set of words, symbols and rules to communicate, while the term *speech* refers to the sequence of articulatory movements (phono-articulatory gestures) culminating in the phonation and articulation of words at the basis of verbal production/comunication.

1.1 LANGUAGE EVOLUTION

The human language as we know it, characterized by grammar rules, is a recent evolutionary achievement: archaeological evidence suggests that it emerged within the past 100000 years with *Homo sapiens* (Tattersal, 2010, Bickerton, 2007). According Derek Bickerton (1926 - today), the

earliest *Homo* (2.3 to 2.4 million years ago) already communicated with a form of "protolanguage", which then underwent further evolution as a result of the behavioural adaptation pressure faced by *Homo habilis* (2.3 to 1.4 million years ago). This ancestral structure of language can be re-called in the so-called "pidgin" languages, i.e. simplified languages used as a means of communication between people not sharing a common language, in the early words that children pronounce, in the symbols used by trained chimpanzees in artificial conditions and finally in utterances of some children affected by in speech delay disorders (Kirby, 2007; Ardila, 2015). A partially different view of language evolution is proposed by Ray Jackendoff (1945 – today) suggesting the succession of several consequent intermediate stages or protolanguages resulting eventually in the complete, complex structure of modern human languages.

Concurrently to the development of the theoretical linguistic models, the evolutionary biologists examined species with a very ancient common ancestor with humans, searching for evidence of convergent evolution, or alternatively, species with relatively recent common ancestor with humans to search for shared features. However, at present none of these two approaches can be adopted to study language, given that no animal species shows a function equivalent to human language. However, some of the essential features of the spoken human language, such as the production of sequences of ordered sounds, show interesting similarities with other species, such as the songbirds. In human and songbirds off springs, individuals imitate the vocalizations of adults during a sensitive period early in life and they go through a "babbling" stage before they reach the adult mature mode (Berwick et al., 2013; Berwick and Chomsky, Why only us: Language and Evolution, 2016). Both humans and songbirds share a vocal organ (i.e. the larynx and syrinx respectively) controlled by central neural pathways to sub-serve different functions as vocalizing, swallowing and airway protection. In both species, the development of motor control of vocal systems emerges from the complex sensory-motor interaction between morphological changes occurring in the developing vocal organ with central neural control mechanisms (Riede and Goller 2010).

Vocal organs. In songbirds, sound is generated by the interplay between the syrinx, the vocal organ located at the caudal end of the trachea, and the respiratory system. Sound results by the airflow through a pair of labia, functionally similar to vocal folds in the human larynx, inducing their vibration. As for the human larynx, the muscle activity of the syrinx sets the oscillating tissues into pre-phonatory position, and the viscoelastic properties of the vibrating tissues determine acoustic output. The human vocal tract is, instead, located in the cranial end of the trachea and it includes different portions of the larynx (sub-laryngeal, intra-laryngeal and supra-laryngeal), the nasal, oral and pharyngeal cavities, all parts of the upper airways. In humans, the area and length of the supra-

laryngeal vocal tract and the nasal tract can be modulated and, accordingly, the pith, the timber and the intensity of the vocal sound are affected (Kirby, 2007). The same mechanism is not found in songbirds. Thus, even if the vocal organs itself (and the respiratory systems) of the two species are similar, the properties of the resonant cavity differ significantly leading in a higher degree of freedom in sound modulation specifically developed in humans (Riede and Goller 2010).

Central nervous system. In humans and songbirds, the central neural circuits controlling vocal production share the same main targets, such as the regulation of airflow, the control of sound frequency. Interestingly songbirds are reported to have human-like left hemispheric dominance in cortical activation during birdsong learning (see below for dominance language hemisphere, Moorman et al., 2012) and show a cortical segregation among the areas devoted to vocal production and those involved in auditory perception. In a comparative approach, it has been suggested that in human Broca's area might be the analogue of the Pre-motor Nucleus in songbirds and the Wernicke's region in human of the Caudomedial Nidopallium in songbirds (Bolhuis et al., 2010, Moorman et al., 2012).

The songbirds and the humans have also genetic common factors, in particular transcription factors (i.e., FOXP2, enhancer) not found in vocal not-learners (for example monkeys and chimpanzees; Pfenning et al., 2014). Despite these very interesting similarities, when considering the cognitive features of human language, the two species –songbirds and human- significantly diverge. The human language is, in fact, composted by hierarchical structures that are assembled by combining words into higher-order phrases and whole sentences (Berwick et al., 2011). In birdsongs, individual notes are also combined as particular sequences into syllables, syllables into "motifs", and the "motifs" into complete song. These elements sequencing in the songs are controlled by rules, called "phonological syntax". However, at present, there is no evidence to suggest that birdsongs can form context-free languages or exhibit the hierarchical structure that characterizes the human language (Beckers et al., 2012). Therefore, the auditory–vocal learning ability evolved in both humans and songbirds, due to a convergent evolution, is not enough to explain the emergence of a complex cognitive structure as that of the human language. The human language has, in fact, a component of externalization of the internal representations, which limits the comparative power of the songbird model (Berwick et al., 2013).

Instead, the comparative phylogenetic studies among the humans and the non-human primates, a specie with relatively recent common ancestor with humans, reveal structural similarities and also differences in brain structures possibly related to language and specifically:

i) the subcortical fibres connecting the cortical areas mainly devoted to language perception and production (see below Wernicke's area and Broca's area, respectively) are more

developed in humans compared to non-human primates, coherently with the observation that the latter do not speak;

ii) the analysis of the oro-facial musculature and the central nervous structures (Sherwood et al., 2003, 2004a, 2005; Diogo et al., 2009), controlling facial movements (speech production), suggests that some facial muscles, such as lower perioral centrifugal muscles, are phylogenetically recent and are more developed in humans compared to non-human primates;

iii) in the non-human great *apes* and humans, the motor cortices innervating the oro-facial muscles are thicker and show more complex local circuitry. In these species the oro-facial muscles are mainly controlled by the primary motor cortex that sends a larger contingent of direct projections to motor nuclei driving oro-facial muscles (Kuypers, 1958a,b; Sherwood et al., 2004a,b), when compared to the other monkeys;

Thus, overall considering the literature on songbirds and non-human primates, the evolutionary biologists conclusively suggest that there is not an animal model that shares with humans a convergent evolution of language development. On one side, the songbirds share with humans a degree of genetic and brain homology, particularly involved in auditory learning and vocal production, on the other side, the motor control of the phono-articulatory apparatus (oro-facial muscles) share interesting similarities with monkeys (Berwick et al., 2013). Given the complexity of the human language function, non-available in animal models, it seems to be adequate to study the neural control of this cognitive human ability only in humans, by means of direct and indirect techniques (direct electrical stimulation, electroencephalography or corticography, functional magnetic resonance imaging) during language tasks.

1.2 HUMAN LANGUAGE

The fundamental feature of human language is that we can produce and understand a boundless number of expressions, intelligible only to others sharing a common knowledge.

The most recent theories on human language claims that it is based on a *computational* mechanism, realized by neural networks, that produces an array of structured expressions. This array depends on the interaction of the genetic endowment with the external input (i.e. environmental stimuli). The genetic potential includes the so-called "universal grammar", or rather the common syntax among all human languages, the constraints ingrained in the brain structure and other cognitive pre-conditions (e.g., the analytical capacity).

The computational theory emerged recently, given that until the first half of the twentieth century the human language was considered a faculty developing on behavioural basis rather than on a computational mechanism. In 1957, in a publication entitled Verbal Behaviour, Burrhus Frederic Skinner (1904-1950) attributed the language to a simple associative mechanism: the human pronounces single words and/or phrases in a determined context due to the experiential positive association between those words and phrases and the same context. Subsequently, Avram Noam Chomsky (1928 – today) proposed the so-called "computational theory of the mind", at present the most accredited. According to this theory, human language is considered:

- a *computational* ability composed by abstract representations as input (categories of information as noun and verbs), calculates relationships between the representations and derives a combinatorial set;
- a *productive* ability: based on a set of categories of information and on computational rules (phonological and semantic), it generates an endless stream of possible combinations (phrase and sentences by means of syntactic rules).

A.N. Chomsky also postulated the existence of an innate substrate underlying human language ability, already existing at birth. According to this model, the new-born has a "pre-loaded" knowledge/cognitive matrix of language, allowing her/him to activate the detailed syntax of all existing human languages. This theory is based on the evidence of a common syntax among all human languages, defined by Chomsky as the "universal grammar" (Hickok, Text-book: The Myth of Mirror Neurons: The Real Neuroscience of Communication and Cognition, 2014). It is in light of this universal grammar common to all languages that emerges the theory of an innate substrate, i.e. an innate brain network underlying both the computational and the productive ability characterizing the human language (Musso et al., 2003). The evolution of this brain network marks the origin of language ability in humans (Berwick et al., 2013).

Linguistic theories within the human brain

To the present knowledge, the neural network underlying the human language ability is composed by cortical and subcortical brain structures involved in: i) the perception of acoustic speech-related inputs; ii) the comprehension of language allowed by matching conceptual and semantic representations; iii) speech production according (or irrespectively) to comprehension; iv) the repetition of words and not-words. Thus, at the neural level, two are the core computational components of language, i.e. conceptual component and a sensory-motor interface, each one consisting in a set of brain areas connected via dedicated systems of connecting fibers and together composing the neural language network.

In the historical classic model, describing the language network, the motor and sensory areas of the brain were in charge of the language production and perception, respectively. Within this model, the motor areas were identified in the inferior frontal *gyrus* and in particular in the region called "Broca's area", while the sensory areas in a region of the superior temporal *gyru*s called "Wernicke's area". In this model, a subcortical *fasciculus*, called arcuate *fasciculus*, connects the sensory with the motor areas to allow the sensory-motor integration fed mainly by auditory inputs (**Fig. 1.1**).

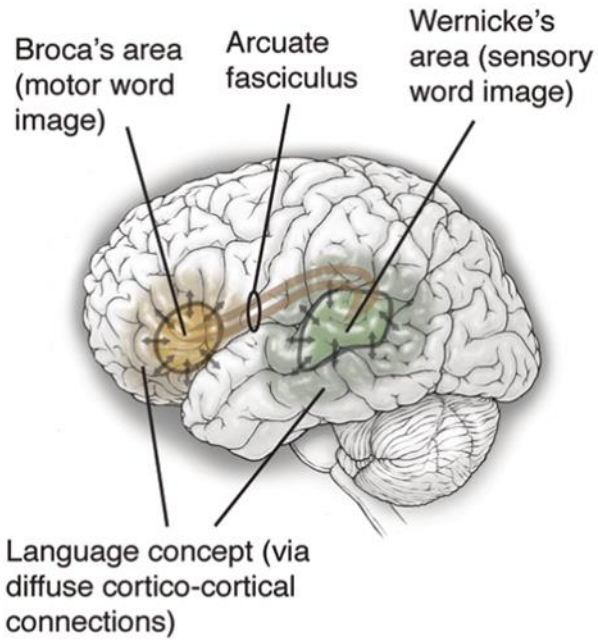

Figure 1.1 Classical model of language organization in the left hemisphere of the brain. Broca's area (gold) is located in the inferior frontal *gyrus* and Wernicke's area (green) in the posterior superior temporal lobe, connected by the arcuate *fasciculus*.

From *Chang et al., 2014*

For more than one century this model was not challenged nor discussed, but the development of the functional neuroimaging techniques and of the intraoperatory brain mapping during resection of brain tumours, by means of direct electrical stimulation, provided new insights on the neural circuitry underlying the language function. Based on the most recent evidence acquired by new techniques, a

new and more modern neural model for language emerged. According to this model, the language function is sub-served by two circuits (or streams) called the "dorsal" and the "ventral" streams (**Fig. 1.2**). The ventral stream involves structures in the inferior frontal lobe, the superior and middle portions of the temporal lobe (Berwick et al., 2013) and portions of occipital lobe (Chang et al., 2015) and is suggested to be involved in processing of visual and acoustic speech-related inputs to allow the comprehension (language recognition). The dorsal stream involves structures in the posterior frontal lobe, the posterior and most dorsal temporal lobe and the parietal operculum and is suggested to have a role in translating acoustic speech-related signals into articulatory gestures in the frontal lobe, a sensory-motor integration needed both for speech development and production. Therefore, the dorsal pathway (blue in **Fig. 1.2**) translates the sensory representations into articulatory gestures, while the ventral pathway (pink in **Fig. 1.2**) translates the sensory or phonological representations into lexical conceptual representations (Hickok and Poeppel, 2007).

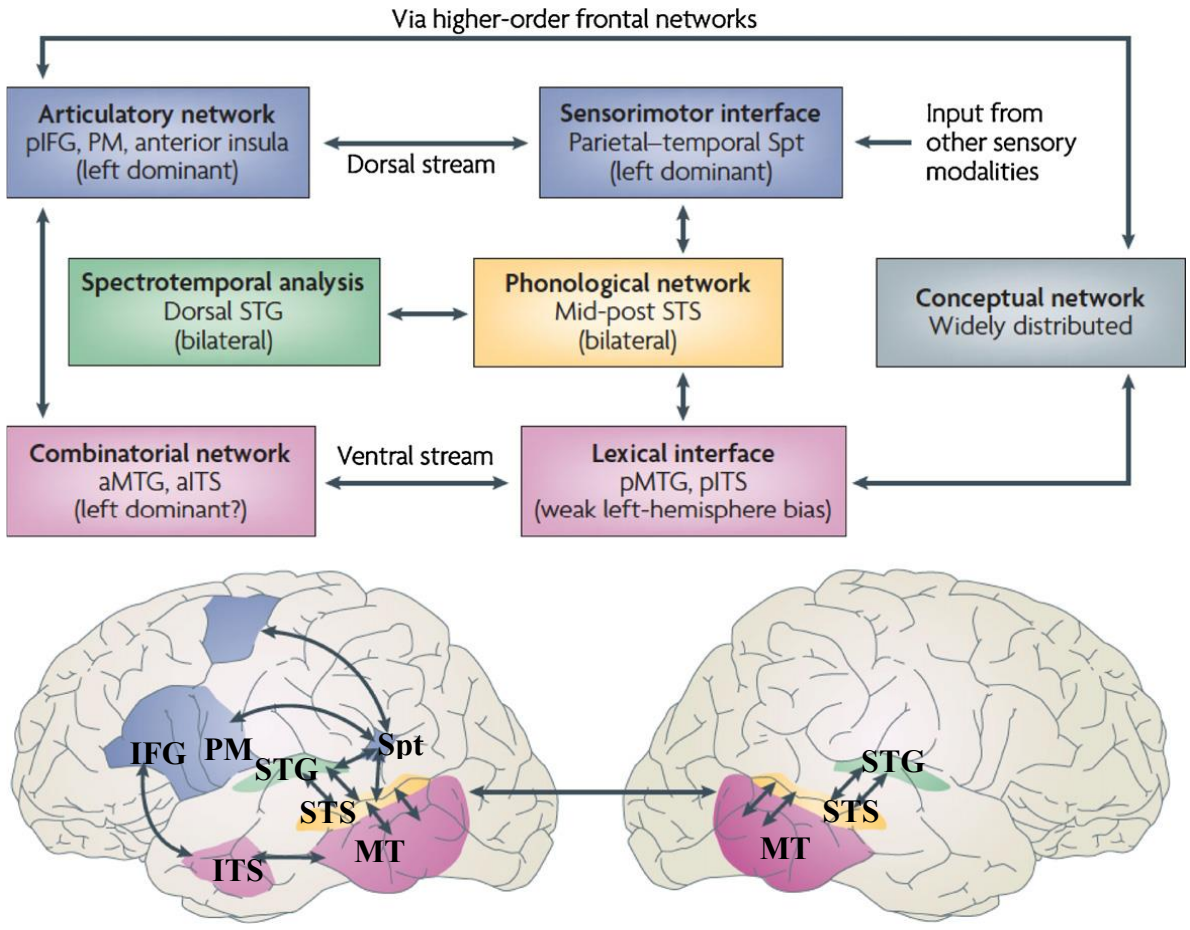

Figure 1.2 Dual stream model of the human language: the modern theory. pIFG = posterior inferior frontal *gyrus*; PM = pre-motor cortex; STG = superior temporal *gyrus*; Spt = sylvian-parietal-temporal area; STS = superior temporal sulcus; MT = middle temporal lobe; ITS = inferior temporal sulcus; MTG = middle temporal *gyrus*.

From *Hickok and Poeppel 2007*, modified.

The Classic Theory

Two centuries ago, the pioneering observations of Paul Pierre Broca (1824-1880) and Karl Wernicke (1848-1905) led in the identification of some of the main cortical areas sub-serving human language ability. These areas were identified, in patients with brain lesions, by matching the site of injury with the occurrence of specific deficits in language function. Due to the scientific significance of their studies, the portion of the inferior frontal *gyrus* supposedly associated with the function of speech production was named Broca's area, while the posterior superior temporal cortex supposedly associated with the understanding of language was named Wernicke's area. More recent studies, however, suggested that this dichotomic results from an excessive over-simplification of the complex mechanisms sub-serving language ability. It was indeed clear, already at the time of P.P. Broca and K. Wernicke's studies, that the lesions in the frontal lobe caused both production and comprehension deficits, and vice versa for lesions involving lesions of the areas of the temporal lobe. Despite an exponential increase in the number of studies on the neurobiology of language over the past 15 years, and the emergence of the computational models of language many fundamental issues, e.g. the exact role of Broca's and Wernike's area in the complex modern model of language network remain, at present, unresolved.

Broca's area

In the 1860s, P.P. Broca, a French surgeon, anatomist and anthropologist, examined a 51-year-old patient with multiple neurological problems, who lost the ability to speak. When attempting to speak, the patient could only produce a single repetitive syllable: "tan". Intonation was not affected. When the patient died, the post mortem inspection of the brain showed a lesion on the surface of the left frontal lobe (**Fig. 1.3**). The association between the lesion and the deficit was presented to the Anthropological Society and to the Anatomical Society of Paris as the evidence that cognitive functions can be localized in specific cortical area of the brain.

The investigation of another patient with similar deficits associated to lesions in the frontal lobe (**Fig. 1.4**) strengthened P.P. Broca's conclusion: "the integrity of the third frontal convolution (and perhaps of the second) seems indispensable to the exercise of the faculty of articulate language". He found in the second patient that "the lesion occupied exactly the same seat as with the first - immediately behind the middle third, opposite the insula and precisely on the same side" (Dronkers et al., 2007).

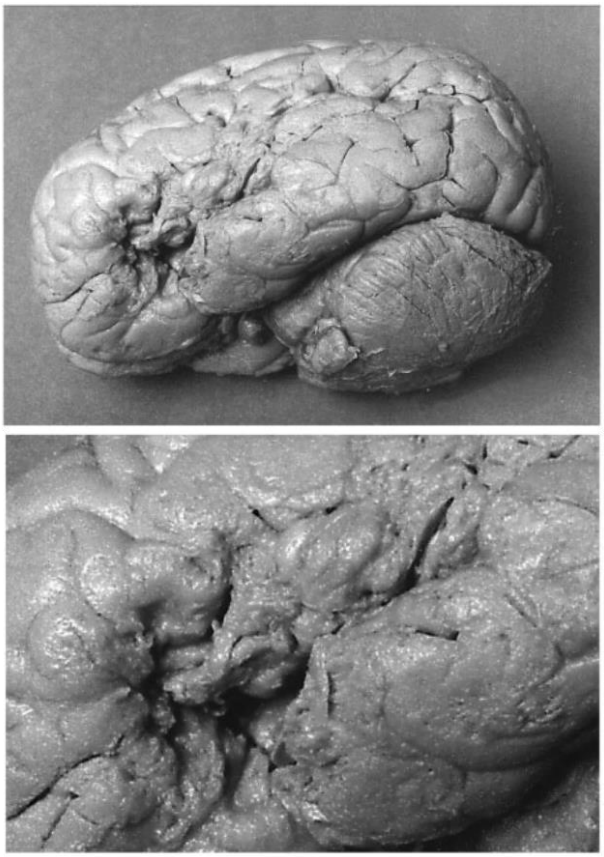

Figure 1.3 Photographs of the brain of Leborgne (first patient). In the pic above: lateral view of the brain. The external lesion is clearly visible in the inferior frontal *gyrus*. The softening in the area superior and posterior to the lesion suggests further cortical and subcortical involvement. In the pic below: close-up of the visible lesion in the brain.

From *Dronkers et al., 2007*, modified.

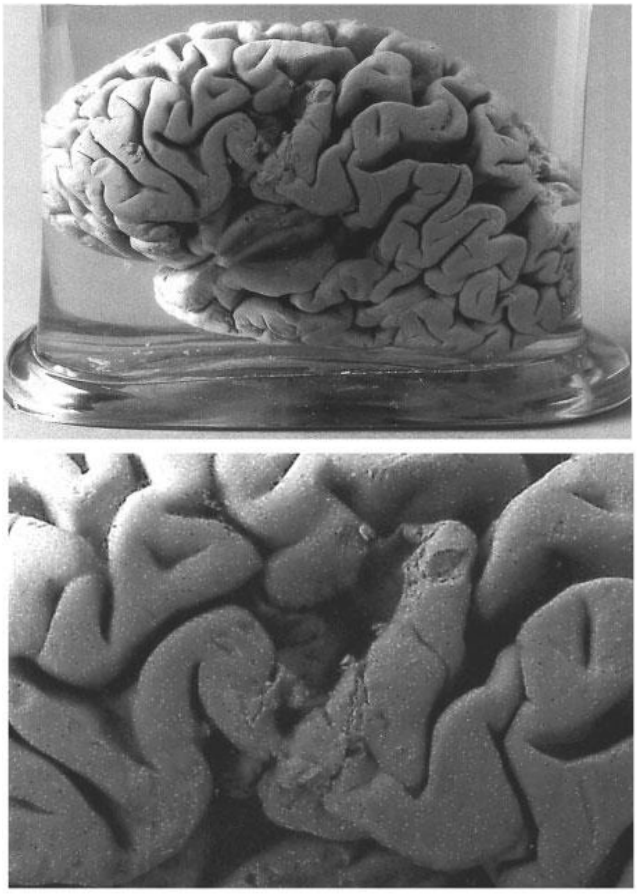

Figure 1.4 Photographs of the brain of Lelong (second patient). In the pic above: the frontal, temporal and parietal lobes have retracted due to severe atrophy, exposing the insula. In the pic below: close-up of the visible lesion in Lelong's brain. Note that only the most posterior part of what is currently called Broca's area is infarcted; the anterior portion is completely spared.

From *Dronkers et al., 2007*, modified.

Since then, the third frontal convolution, at present anatomically defined the *pars opercularis* and *pars triangularis* of the inferior frontal *gyrus* (Brodmann's (1909) areas 44 and 45: BA44-BA45), was considered the cortical region controlling the motor articulation of language (Fridriksson et al., 2015). This finding demonstrated, importantly, that the left hemisphere was dominant for the language articulation and since then, all the deficits in language production following the lesion of Broca's area, patients were affected by so-called *Broca's* or *production* aphasia (Dronkers et al., 2007). Years later, it was demonstrated that, despite the lesion of Broca's area was needed to induce Broca's aphasia, other brain structures such as the insula, parietal regions, and their underlying white matter might be involved. Although the attribution of Broca's aphasia to a pure lesion of Broca's area has been highly debated, no doubts have been arised on the critical role of this area in language production so far (Fridriksson et al., 2015).

Wernicke's area

Soon after P.P. Broca made his revolutionary observations, Karl Wernicke, a German physician, anatomist, psychiatrist and neuropathologist, suggested the cortex located posterior to the central sulcus to be responsible for the language-related sensory functions. This hypothesis was supported by the observation that lesions in the posterior superior temporal lobe were associated to paraphasic errors with impaired naming, repetition and comprehension of words, but preserved speech fluency (Chang et al., 2015). Moreover, K. Wernicke suggested the involvement of the frontal and the temporal areas of language in the memory storage of motor and sensory representations and accordingly nowadays, Broca's area was suggested to host the "motor images of speech", while the posterior superior temporal cortex, named Wernike's area (Brodmann area 22, BA22), the "acoustic images for words" (Hickok and Poeppel, 2004). According to this view, K. Wernicke postulated a connection, by means of subcortical fibres (**Fig. 1.1**), between the two areas, firstly needed in postnatal language development acquisition. According to this theory, the child, upon hearing a word or syllable, reflexively mimics that same word/syllable in speech. This in turn would cause the simultaneous activation of the corresponding sensory and motor representations in the cortex, leading eventually in a direct reflex association occurring via a cortico-cortical pathway running behind the insular cortex (**Fig. 1.1** and **1.5**).

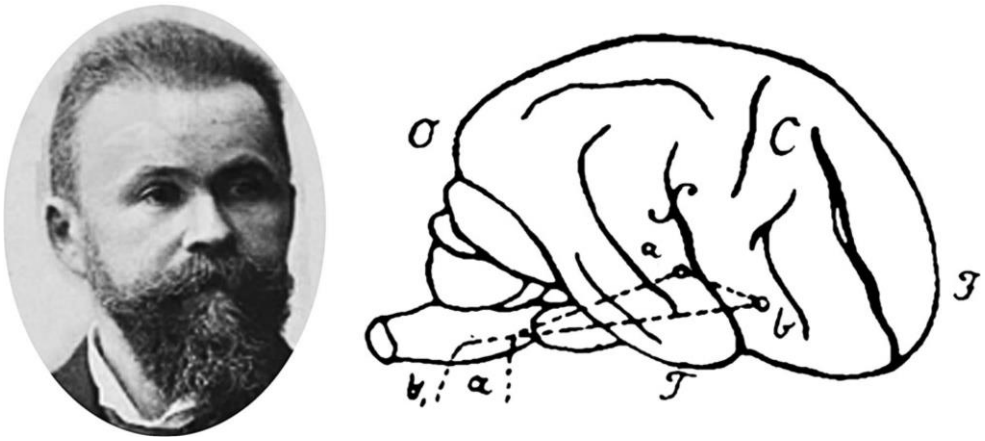

Figure 1.5 K. Wernicke (1848−1905) and his representation of the language network from his 1874 MD thesis. From *Catani and Mesulam, 2008*.

According to K. Wernicke, these sensory (acoustic) and motor representations of a word were distinct from the concepts with which they were associated: while the acoustic representation of a word was a purely auditory entity, the concept emerged as the sum of all the representations stored in memory and associated with it: understanding the meaning of a word implied the association between the incoming acoustic representation and all the memory representations of the related concept. In such a model, an anatomical connection between the two cortical language centres must be paralleled by a system connecting the conceptual representations. The spontaneous production involved the arousal of a conceptual representation, which feeds the corresponding motor and sensory representations of the concept to be translated in sounds. In such a model, despite the degree of overlap between language perception (sensory) and production (motor), lesions in different areas of the system were expected, and actually confirmed by the clinical observations, to result in different deficits: lesions of the frontal areas produced a deficit in the production of speech leaving intact the language comprehension (*production* or *Broca's aphasia*), while lesions to temporal cortex lead in deficits in the comprehension (*sensory* or *Wernicke's aphasia*). An "intermediate" deficit results instead from the disconnection of the fibres connecting frontal and temporal areas (see **Fig. 1.5,** *conduction aphasia*), characterized by repetition errors with intact fluency and comprehension (Chang et al., 2015). A more detailed explanation of the conduction aphasia was given by Norman Geschwind (1926-1984), an American behavioural neurologist, who suggested the involvement of a specific group of fibres connecting Wernicke's area with Broca's area, called the arcuate *fasciculus* (see **Fig. 1.6**, Chang et al., 2015). Based on these anatomical findings, the Wernicke-Geschwind language

model was proposed claiming that upon hearing a word, a sensory word representation was created in Wernicke's area. The meaning behind the word (the conceptual representation) was deduced from the interaction among different connections originating from the language centres. Simultaneously to the sensory representation, a motor word representation emerged in Broca's area as a result of the reflexive cortico-cortical connections between the two primary language areas, thus the acoustic image was essential for the selection of the proper motor word image. According to this model, the spontaneous production of language re-called the concept, which then sequentially activated the sensory and motor word representations.

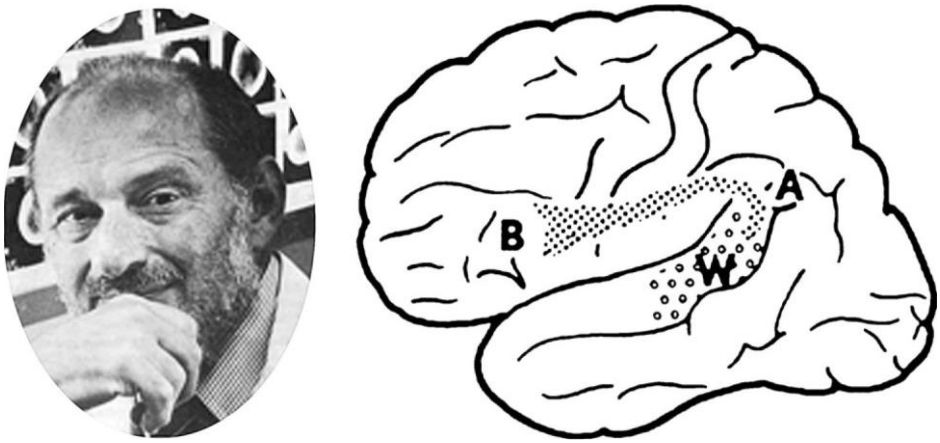

Figure 1.6 Norman Geschwind (1926−1984) and his representation of the language network from his 1970 Science paper. B: Broca's area; W: Wernicke's area; A: arcuate f*asciculus*.

From *Catani and Mesulam, 2008*.

Criticisms on the Classical Theory

P. P. Broca and K. Wernicke gave a priceless contribution in the investigation of neural circuits underlying language ability, the classical model emerging from their studies is still not comprehensive for the following issues.

a) It does not clarify how the phonological (sounds), lexical (words), semantic (meaning of words and sentences) components of language are processed nor where and how the syntactic processes are computed.

b) It does not explain the evidence that Broca's, Wernicke's and conduction aphasia do not occur solely as a result of pure lesions of Broca's and Wernicke's area and the arcuate *fasciculus* respectively, but are also observed following lesions of other brain structures:

- Broca's aphasia. Studies on pure lesions of Broca's area failed to associate production aphasia to the lesion and rather reported a transitory, rapidly improving mutism (Mohr et al., 1978). This observation was supported by the neuroradiological analysis (high resolution MRI analysis, see Dronkers et al., 2007) of the preserved brains of P.P.Broca's patients, showing that the lesion was not confined to the inferior frontal *gyrus* (**Fig. 1.7**), but involved also the inferior parietal lobe, the anterior superior temporal lobe and the insula, with many subcortical structures, including the basal ganglia (claustrum, putamen, globus pallidus, head of caudate

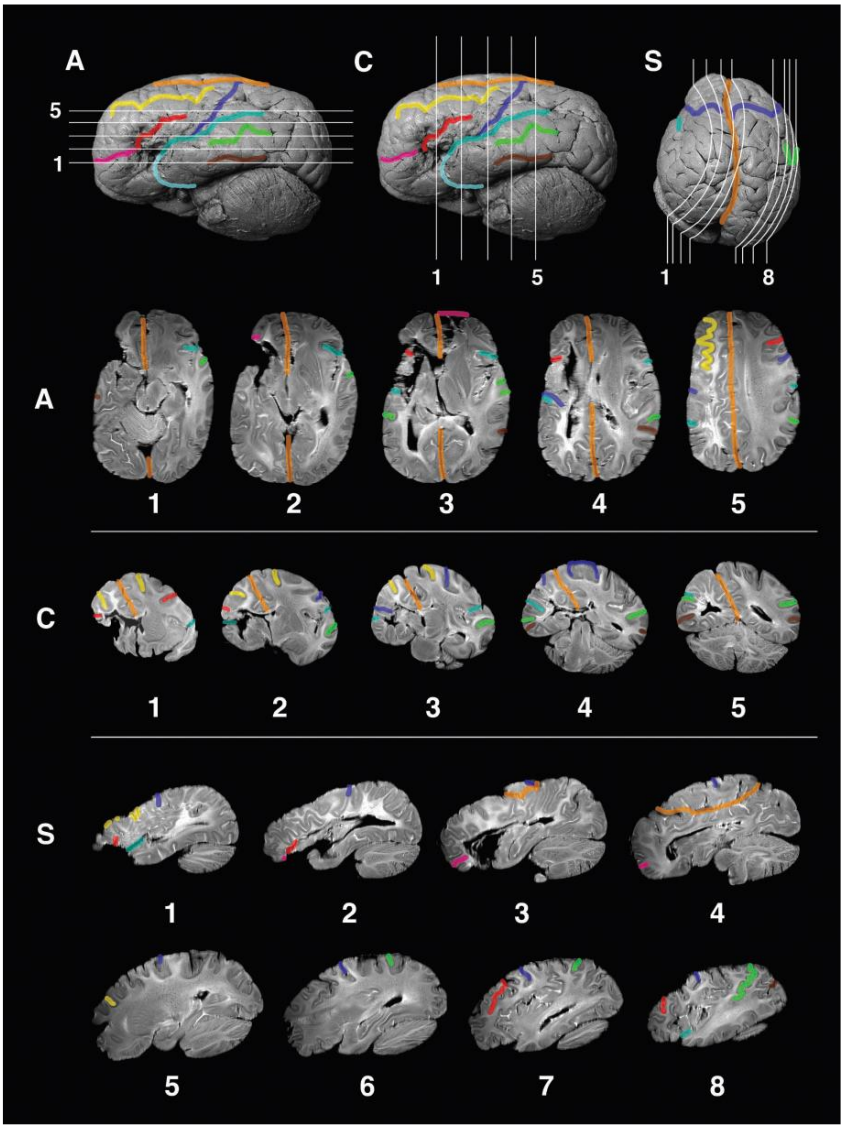

Figure 1.7 High-resolution MRI of the preserved brain of Leborgne with representative slices throughout the brain. The first row shows the photographs of the lateral and superior surfaces of the brain, with lines indicating the slices shown below. Row **A** shows axial slices, Row **C** coronal slices, and Row **S** sagittal slices through the left and intact right hemisphere for comparison with each other. In the axial and coronal planes, the left hemisphere appears on the left side of the images. The following structures are delineated: interhemispheric/longitudinal fissure (orange), central sulcus/Rolandic fissure (dark blue), sylvian/lateral fissure (aqua), inferior frontal sulcus (red), superior frontal sulcus (yellow), frontomarginal sulcus (pink), superior temporal sulcus (light green) and inferior temporal sulcus (brown). Sagittal slices S3 and S4 show the superior portion of the right hemisphere crossing over the midline due to extensive damage in the left hemisphere.

From *Dronkers et al., 2007*.

nucleus), internal and external capsules and other white matter tracts (superior longitudinal fasciculus, medial subcallosal fasciculus). In light of this finding, the aphasia described by P. P. Broca, as a permanent loss of the ability to articulate language, might be due to a lesion of anatomical regions different from Broca's area but adjacent to it and focused the attention on deep grey matter, and in particular the thalamus and the basal ganglia, previously not believed to be involved in the control of language function (Dronkers, 2000).

- Wernicke's aphasia. It has been observed that Wernicke's aphasia, follows pure lesion of Wernicke's area (BA22), as well as lesions to the posterior medial temporal *gyrus* and underlying white matter, the anterior superior temporal *gyrus*, the superior temporal sulcus and angular *gyrus*, and two prefrontal areas (BA46-BA47) (Dronkers et al., 2004).
- Conduction aphasia. Recent evidence showed that it is not caused by damage to arcuate *fasciculus* and appears not to be actually a disconnection syndrome. Perception of language seems to be computed bilaterally in the brain (Hickok et al., 2008).

The Modern Theory

Recently, a new linguistic organization model, overcoming some of the limitations of the classical theory, has been proposed. This modern model accounts for the production, the comprehension and also the complexity (from phonology to syntax) of language.

The human language is in fact composed of three components: the syntactic rules and representations, the sensory-motor interface, and the internal conceptual-intentional interface (Berwick et al., 2013; **Fig. 1.8**).

The syntactic rules and representations. The syntactic rules and representation levels constitute the basis of the language system. The levels of representation are: i) the phonological level, where the sequences of sounds are checked; ii) the syntactic level, where words are combined yielding the proper hierarchical structures; iii) the semantic level, where the meaning of the whole sentence is computed on the basis of the meaning of each lexical item (Moro et al., 2001). The mental expression emerges once the three levels are all computed.

External sensory-motor interface. Through the sensory-motor interface, the mental expressions are transmitted to the external world. Once the pre-lexical intention is transformed into lexical units (words), temporally sequenced and coded on the phonological, syntactical and semantic levels (Levelt et al., 1999), the sensory-motor system produces words by organizing the movement of the phono-articulatory apparatus. The latter is composed of the about 100 muscles used for verbal

communication as well as for other functions (e.g. grasping, mastication and communicative expressions. Kent, 2000).

Internal conceptual-intentional interface. The internal conceptual-intentional interface connects the mental expressions to semantic-pragmatic interpretation, reasoning, planning, and other activities of the internalized "mental world", inasmuch the human language serves primarily as an internal instrument of thought.

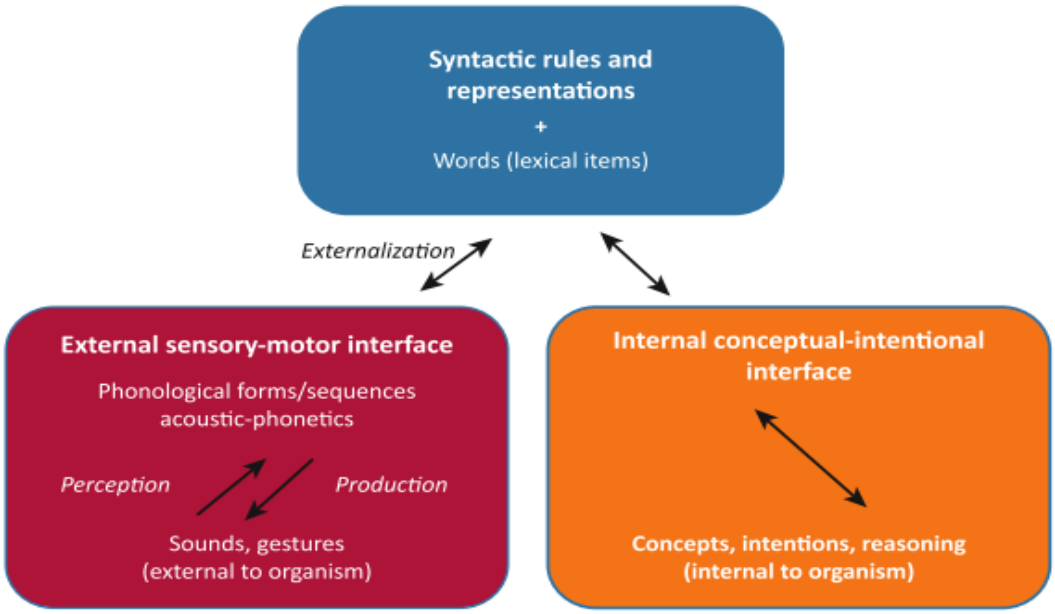

Figure 1.8 Theoretical model of the human language.

From *Berwick et al., 2013*.

The modern model of neural network underlying emerges very similar to the "dual stream" model proposed, and widely accepted, to explain the visual system, i.e. the so-called "dual stream model".

Dual stream model

Dual stream in the visual system. The visual system is functionally organized in two main streams: the dorsal and the ventral stream, named the "where" and "what" pathway (Mishkin et al., 1982) respectively, due to their functional role in processing visual information computed in the occipital visual cortex. In the dorsal stream, the occipital visual cortex projects to parietal areas to sub-serve the sensory perception regarding the object location (the so-called "where" pathway), needed to visuo-motor integration (Andersen, 1997; Rizzolatti et al., 1997); while in the ventral stream, the

occipital visual cortex projects to the inferior temporal areas and it is involved in processing object identity (the "what" pathway, Hickok and Poeppel, 2004).

Dual stream in the language system. The dual stream model of language emerges from the observation that language needs a double processing of acoustic inputs: first verbal speech conveyed by acustic input must be understood, i.e. linked to conceptual-semantic representations (ventral stream) and, second, acoustic inputs are needed to sensory-motor control of speech production (dorsal stream). The "segregation" of these two pathways is demonstrated by the fact that humans can translate a sound into phono-articulatory gestures without a mandatory involvement of the conceptual system, e.g. during pseudo-word repetition. Moreover, in acquired or congenital neurological disease or temporary deactivation of the motor system controlling the ability to actually perform speech, the comprehension of the spoken language in not necessarily affected/impaired (Hickok, 2012).

In 2000, Hickok and Poeppel were the first to hypothesize the cortical dual stream model sub-serving the linguistic process, mostly related to single word tasks. According to this model, upon hearing a word, the Superior Temporal *Gyrus* (STG, in red in **Fig. 1.9**, Hickok and Poeppel, 2000), including the Superior Temporal Sulcus (STS), is activated bilaterally to perform a spectro-temporal and phonological analyses of the incoming speech sounds (Chang et al., 2014). The STS elaborates all the phonological information (Indefrey and Levelt, 2004; Hickok and Poeppel, 2007). This site

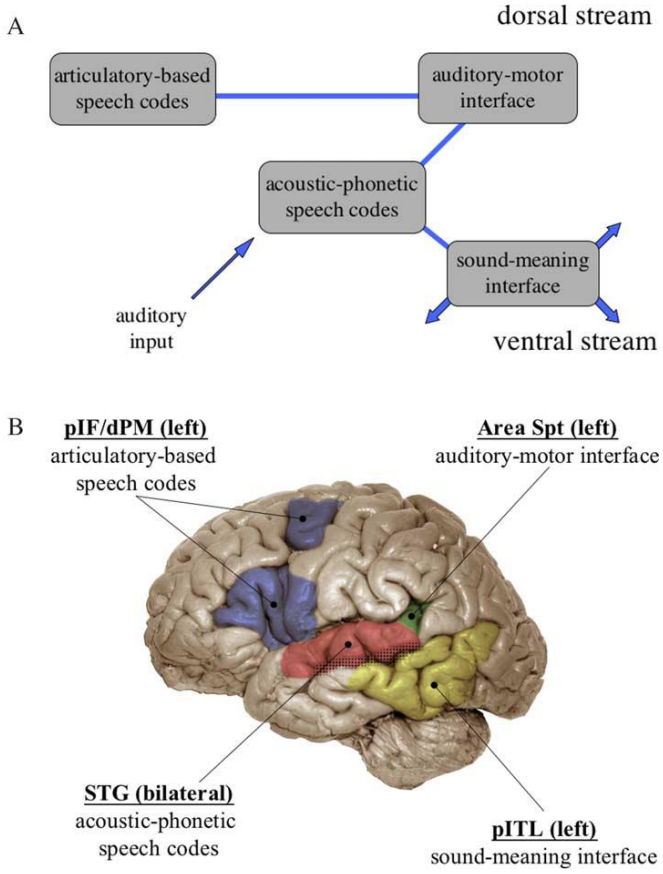

Figure 1.9 In the panel **A**: the language model proposed by Hickok and Poeppel. In the panel **B**: lateral view of the left hemisphere. The frontal areas (blue areas) thought to support articulatory-based speech codes comes from functional imaging studies of object naming and articulatory rehearsal processes. The stippled area (superior temporal sulcus) represents a region, which appears to support phoneme-level representations
pIF = posterior Inferior Frontal *gyrus*; dPM = dorsal PreMotor cortex; STG = Superior Temporal *Gyrus*; pITL = posterior Inferior Temporal Lobe; Area Spt = Sylvian-parietal-temporal area.

From *Hickok and Poeppel, 2004*.

represents the origin of the two streams: the ventral stream, involved in mapping sound into meaning, and the dorsal stream, using sounds to control the phono-articulatory gestures (Hickok and Poeppel, 2000).

The *ventral stream* projects ventral-laterally, involving the STS and the STG (**Fig. 1.2**), and ultimately the posterior Inferior Temporal lobe (pITL, i.e. portions of the Middle Temporal *Gyrus* (MTG) and Inferior Temporal *Gyrus* (ITG)). The pITL serves as interface between sound-based representations of speech, perceived in STS-STG, and the conceptual representations (sound-to-meaning interface, i.e. the lemma level of representation). Despite being well represented in both the hemispheres, the ventral stream appears to be left-dominant.

The *dorsal stream* projects dorsal-laterally from the STS to the posterior frontal lobe (in blue in **Fig. 9**) and particularly to the Broca's area, the frontal operculum, the insular cortex, the oro-facial representation in primary motor area, all involved in speech production. The dorsal stream is indeed believed to control the sensory-motor integration needed to translate the acoustic information into phono-articulatory gestures (motor representations, see above). Critical region for sensory-motor integration is the Sylvian–parietal–temporal area (Spt), located deep in the posterior portion of Sylvian fissure between the parietal and temporal lobes.

HSFC model

Language perception. The language perception is needed to understand the other's verbal communication and to control our personal ongoing speech performance (The Handbook of Speech Production, First Edition. Edited by Melissa A.Redford. John Wiley & Sons, Inc. Published 2015), i.e. words, syllables, as well as the meaning conveyed by them (Levelt, 1989). The auditory cortex plays an important role in perception of verbal language: congenitally deaf children cannot speak (Smith, 1975; Oller and Eilers 1988). However, the auditory feedback is not the only type of sensory feedback that the CNS (central nervous system) receives, given that the number of phonemes that we produce in one second (Levelt, 1999) is beyond the computational rate of the auditory system. The CNS receives, in fact, also the somatosensory feedback (proprioceptive mainly) from the phono-articulatory apparatus. However, an impairment of the somatosensory feedback, although affecting phono-articulatory gestures (e.g. lip rounding and fricative constrictions), is not sufficient per se to totally prevent speech output (Scott and Ringel, 1971; Fucci et al., 1977). Thus, both the acoustic and somatosensory feedbacks are needed to the sensory-motor system to control of the speech production. These mechanisms are incorporated in so-called Hierarchical State Feedback Control (HSFC) model (Hickok, 2012a, **Fig. 1.10, 1.11**). The architecture of the model incorporates the levels of language processing. The articulatory controller (frontal cortical regions) sends the motor commands to the

vocal tract and an efferent copy to the internal model, which, in turn, generates predictions as to the state of the vocal tract in the phonological system, as well as predictions of the sensory consequences of phono-articulatory action in the auditory phonological system. The integration between the auditory and motor systems is achieved by an auditory–motor translation system. The predicted

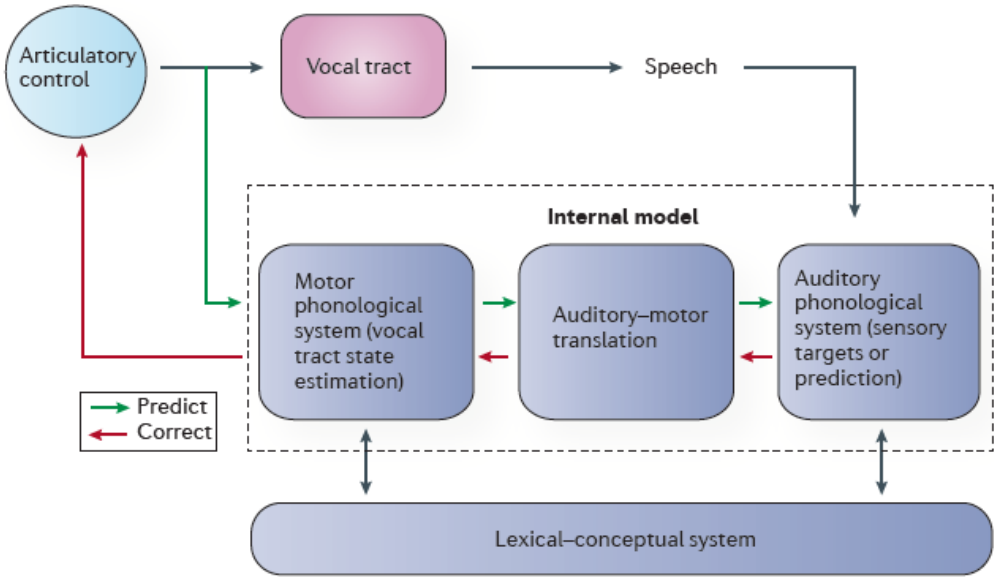

Figure 1.10 The architecture of the state feedback control model.

From *Hickok, 2012*.

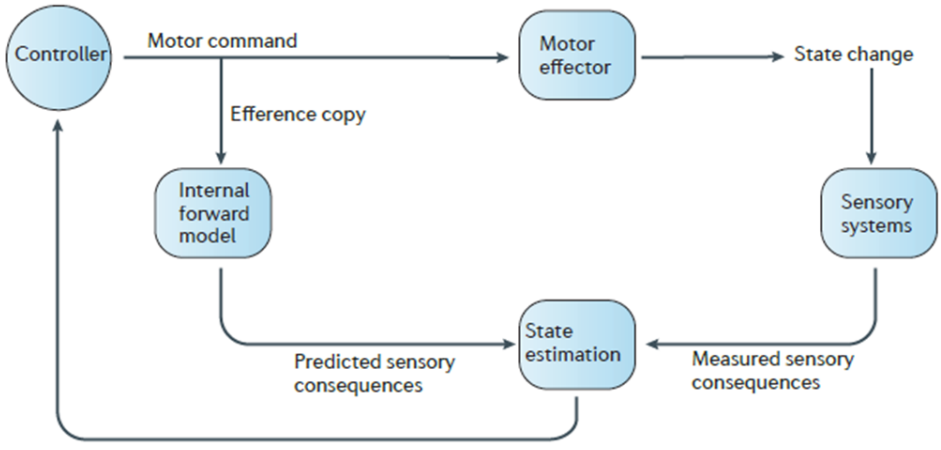

Figure 1.11 State feedback control model includes a motor controller that sends commands to a muscle effector, which in turn results in a change of state (such as a change in the position of an arm). State changes are detected by sensory systems. The model also includes an internal forward model that receives a copy of the motor command that is issued by the controller and generates a prediction of the sensory consequences of the command that can be compared against the measured sensory consequences. The difference between the predicted and measured sensory consequences is used as a motor correction signal that relays to the controller.

From *Hickok, 2012*.

feedback (internal forward model) is then matched with the ongoing somatosensory feedback to control the phono-articulatory ongoing activity. The motor controller is driven by the internal model receiving, in addition to somatosensory inputs, the auditory input (**Fig. 1.10**), the most relevant feedback relative to the product of the phono-articulation and, therefore, used to update the internal model in case of persistent mismatches between the predicted and measured states to detect speech perturbations.

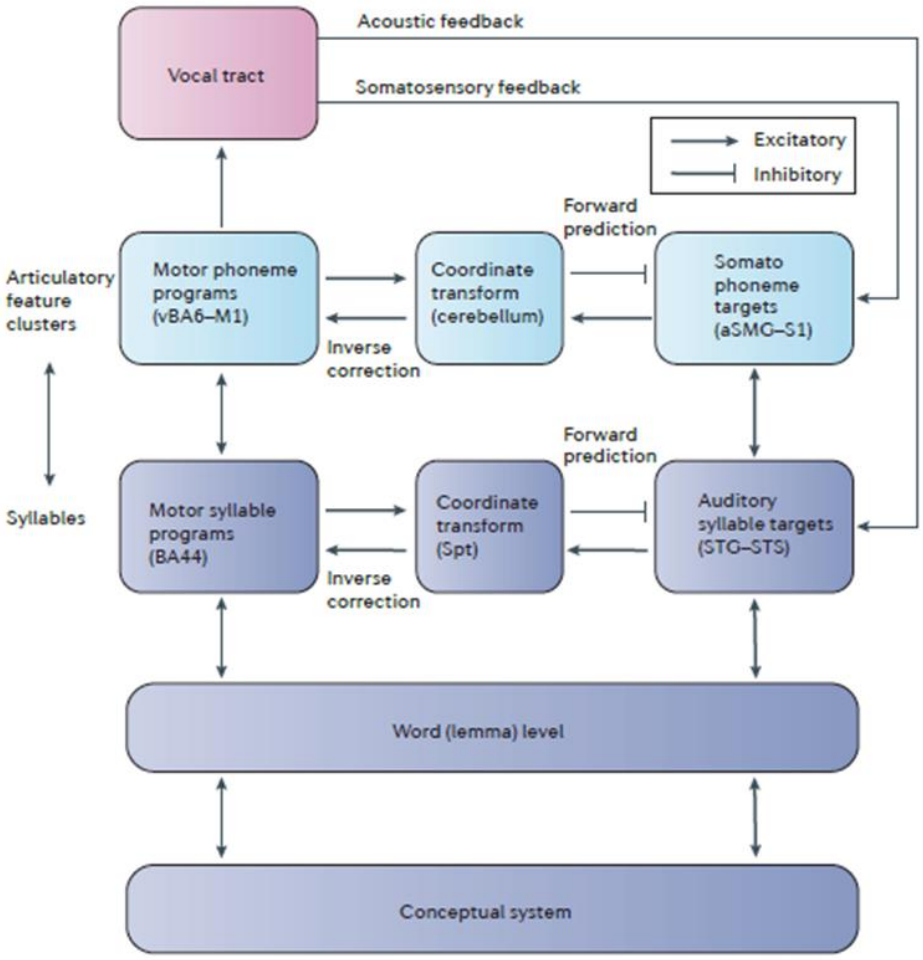

Figure 1.12 The hierarchical state feedback control (HSFC) model includes two hierarchical levels of feedback control. The input to the HSFC model starts with the activation of a conceptual representation that in turn excites a corresponding word (lemma) representation (purple fields). The word level projects in parallel to sensory and motor sides of the highest, fully cortical level of feedback control, the auditory–Spt–BA44 loop. This higher-level loop in turn projects, also in parallel, to the lower-level somatosensory–cerebellum–motor cortex loop (light blue fields). Direct connections between the word level and the lower-level circuit may also exist, although they are not depicted here. The function served by an efference copy is integrated into the motor planning process.
aSMG = anterior supramarginal *gyrus*; M1 = primary motor cortex; S1 = primary somatosensory cortex; STG = superior temporal *gyrus*; STS = superior temporal sulcus; vBA6 = ventral BA6.

From *Hickok, 2012*.

The input to the HSFC model starts with the activation of a conceptual representation that in turn excites a corresponding word (lemma) representation (**Fig. 1.12**). The word level projects in parallel to sensory and motor sides of the highest, fully cortical level of feedback control, the STG-STS–Spt–BA44 loop. This higher-level loop in turn projects, also in parallel, to the lower-level somatosensory–cerebellum–motor cortex loop (Hickok, 2012; Houde and Chang, 2015).

The Motor Theory

In the second half of the XX century, Alvin Liberman (1917 – 2000) proposed the "motor theory of language perception". According to this theory (Liberman et al., 1967; Liberman, 1985), human perception of language occurs through the perception of the motor gestures needed to produce speech and not through the perception of the language sounds. The motor gestures are the phonetic gestures of the speaker, hosted in the brain as "invariant" (constant) motor commands driving movements of the phono-articulators through predetermined linguistically-significant configurations. These commands correspond to elementary movements as for example: tongue backing, lip rounding, and jaw raising, providing the basis for the phonetic gestures. For example, [b] consists of a labial stop gesture, while [m] is produced by the same gesture combined with a velum-lowering gesture. If these motor gestures are invariant, for example, in production of a syllable (as /da/) the single phonetic gestures ([d] and [a]) are produced by invariant motor commands, in the acoustic perception of the syllable (/da/), our perceptive system does not perceive the phones ([d] and [a]) separately, but in parallel (http://www.haskins.yale.edu/featured/patplay.html; Liberman et al., 1967). These different perceptions put the basis of the "motor theory of language perception" (Hickok G. The Myth of Mirror Neurons: The Real Neuroscience of Communication and Cognition. WW Norton and Company. New York London 2014).

Although very fascinating "motor theory of speech perception" was challenged by studies on lesions. According to this theory, the motor component of the language circuit was essential for language perception, therefore all the neurological deficits impairing speech production were expected to impair also language perception. In 1962 Eric Lennberg, a German neurologist, reported the case of a child affected by a syndrome, known today as Foix-Chavany-Marie syndrome: the child was not able to speak, not even after many rehabilitative sessions with a speech therapist, but he was able to understand spoken language without apparent deficits. It has been now demonstrated that the syndrome is due to a bilateral damage of the anterior portion of the frontal operculum associated to paralysis of the motor pathways controlling the voluntary oro-facial movement, without affecting language perception (Hickok G. The Myth of Mirror Neurons: The Real Neuroscience of

Communication and Cognition. WW Norton and Company. New York London. 2014). Moreover, in patients affected by a deficit in speech production is preserved the ability to discriminate among different language sounds (Bishop et al., 1990) challenging the "motor theory of speech perception". Conversely, this theory received a strong support by the discovery of the mirror neurons. In 1992, Di Pellegrino and colleagues described a special population of neurons located in the monkey ventral pre-motor cortex (named F5 region). These neurons were active when the monkey was performing an action involving the hand or mouth district and when the monkey was solely observing the same action (*mirror neurons*). In humans, the observation of others' actions induces a subliminal activation of motor pathways (motor resonance) supposedly mediated by the mirror neuron system and reflects the motor program encoding the observed action. By reproducing a known neural pattern in the motor system of observers, motor resonance directly encodes the observed action and mediates the immediate understanding of the action and its intention (*action understanding*). On this conceptual basis, some authors hypothesized that similar neurons would support the "motor theory of language perception": humans, it was proposed, are able to understand the speech of others by means of a population of mirror neurons which were active also during one's own speech production, the understanding of others speech would then relay on the simulation of speech within the listener's own mind. Based on the assumption that the human analogue of monkey F5, hosting mirror neurons is Broca's area, it was suggested that human language originated by human mirror neurons in Broca's area. This very interesting theory, however, received lots of criticism. Before the discovery of mirror neurons actually nobody suggested an homology between the monkey F5 and Broca's area: although they are cyto-architectonically similar, Broca's area is a cortical region significantly involved in speech production, an ability lacking in monkey. Neuroimaging studies (PET, Rizzolatti et al., 1996) demonstrated that when the humans observe speech actions performed by others, the activity of Broca's area increases, suggesting its involvement in comprehension of the phonetic gestures. However, the component of Broca's area activating in action observation (*pars triangularis* or BA45, Grafton et al., 1996) seems not to be the same activating in execution (*pars opercularis* or BA44) nor in the actions imagery (Grafton et al., 1996). A multidisciplinary study in 2015 demonstrated that the mirror neurons system in human is reasonably located in the ventral pre-motor cortex (Cerri et al., 2015). Furthermore, patients with lesions of Broca's area show impairment of speech production and sometimes in action comprehension, but perception of language is not affected. All these studies, by challenging the homology with Broca's area, an area actually lacking of a proper motor output consequently challenge the "motor theory of speech perception".

The language network

Overall considering the entire attempt to suggest a model of language circuit, the dual stream model enriched with the HSFC model, appears to be the most reasonable and comprehensive among all those proposed.

In the last decade, neuroimaging (with PET, fMRI, Moore and Price, 1999; Van Turennout et al., 2000; Cerri et al., 2015) and advanced electrophysiological studies (Ojemann et al., 1989; Schaffler et al., 1993; Levelt et al., 1999; Salmelin et al., 2000; Tate et al., 2014; Flinker et al., 2015) improved the knowledge of the cortical and subcortical components of human language network, allowing to identify the brain areas activated during production and perception of language and to describe the precise time course of their activations.

Time course of human language in cortical areas

The time course of human language network during speech of single words is subdivided in subsequent phases: i) the *conceptual level*, involving the lexical concepts, i.e. the concepts associated to specific words; ii) the *lemma level*, involving the syntax of the words; iii) the *form level*, involving the linguistic rules to create the words; and iv) the *motor execution* of the phono-articulatory gestures needed to pronounce the words.

Conceptual level

The production of a word starts with the activation of the lexical concept related to it and its selection. During picture naming, for example, the depicted object must be recognized and the corresponding concept selected. Interestingly, multiple lexical concepts are activated in response to a single picture. In **Figure 1.13** an example of the conceptual processing needed to the execution of a naming task is depicted: the picture of a sheep activates the concept "sheep", as well as "animal" or "goat". Which concept will be selected for the expression depends on the communicative situation (in ecological conditions) or the experimental task. In a naming task, the basic level concept (sheep) is selected while in a categorization task, the superordinate concept (animal) will be selected. This strategy is called "perspective taking". The speed of selecting the picture-related concept is, thus, not fixed. The choice of an expression is a rhetorical decision. In a simple naming task, the average time needed from the presentation of the picture to access to the lexical concept is about 150-200 ms (Thorpe et al., 1996; Schmitt et al., 2000), (**Fig. 1.14**). This phase corresponds to the activation of the middle part of the left middle temporal *gyrus*.

Lemma level

Following the conceptual preparation, the construction of a syntactic frame, i.e. grammatical encoding, is mandatory. The sequential order in which the words will be pronounced depends on the syntactic properties of the chosen lexical items. During the lemma selection, different syntactic properties are taken into account, such as for example: word category, gender of nouns, syntactic argument, and structure of verb. In a go/no-go event-related potential study (Schmitt et al., 2001), the timing of lexical selection, in naming task, has been estimated to occur at a delay of about 150- 200 ms with respect to picture presentation (i.e. following the concept selection) and to be concluded between 250 and 350 ms after picture onset (**Fig. 1.14**). This phase corresponds to the activation of the middle part of the middle portion of the left middle temporal *gyrus* (**Fig. 1.15**).

Form level

The range of operations involved in construction of the words begins with accessing the target word's phonological code and ends while the word being articulated.

Phonological code and morpheme's code. Since, a phoneme is a sound unit and a morpheme is the smallest meaningful unit of language, the access to a morpheme's code must be preceded by the access to its phonological segments. It has been estimated that the first phonological segment is selected within 40 and 113 ms following the lemma selection (Van Turennout et al., 1999) (**Fig. 1.14**).

Phonological encoding. The phonological encoding, in speech, involves the syllabification and the metrical encoding. The syllabification is an incremental process, in which the spelled-out segments of the phonological code are clustered in syllabic patterns. Therefore this process is not fixed in the lexicon, but it is produced 'on-line' in a context-dependent fashion. The segment-by-segment internal syllabification of a word proceeds at a speed of about 25 ms per segment (Van Turennout et al., 1997) (**Fig. 1.14**). This phase is associated to activation of the left inferior frontal *gyrus*, i.e. Broca's area (**Fig. 1.15**).

Phonetic encoding. The produced syllables are rapidly turned into motor actions. Motor commands/programs for the few hundreds of high-frequency syllables performed in normal speech must be readily available and, thus, are stored in a mental repository of articulatory syllable scores called the 'mental syllabary'. A precise time course of phonetic encoding is lacking in the literature, mainly due to a significant constraint preventing the possibility to measure this phase independently form the others: the articulation of a plurisyllabic word can be indeed initiated before of the completion of phonetic encoding (**Fig. 1.14**). The phonetic encoding phase is associated to the activation of in the left inferior frontal *gyrus* and precisely to the more posterior and ventral portion

of Broca's area: the ventral sector of BA44 (Hickok and Poeppel 2004; 2007; Guenther et al., 2006; Papoutsi et al., 2009).

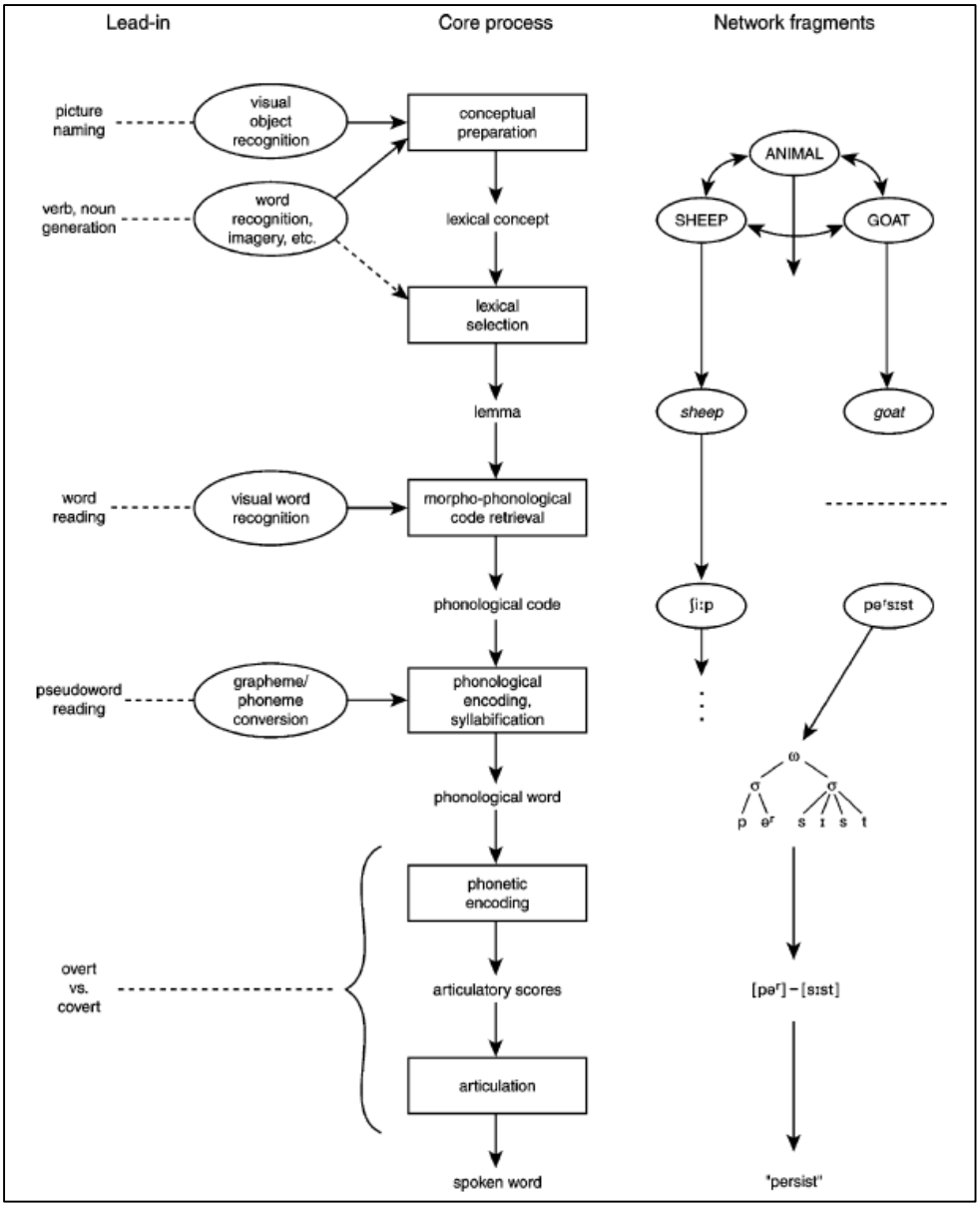

Figure 1.13 Processing language network and componential task analysis. Left column: experimental tasks. Middle column: core processes of word production and their characteristic output. Right column: example fragments of the activation network and its output.

From *Indefrey and Levelt, 2004*.

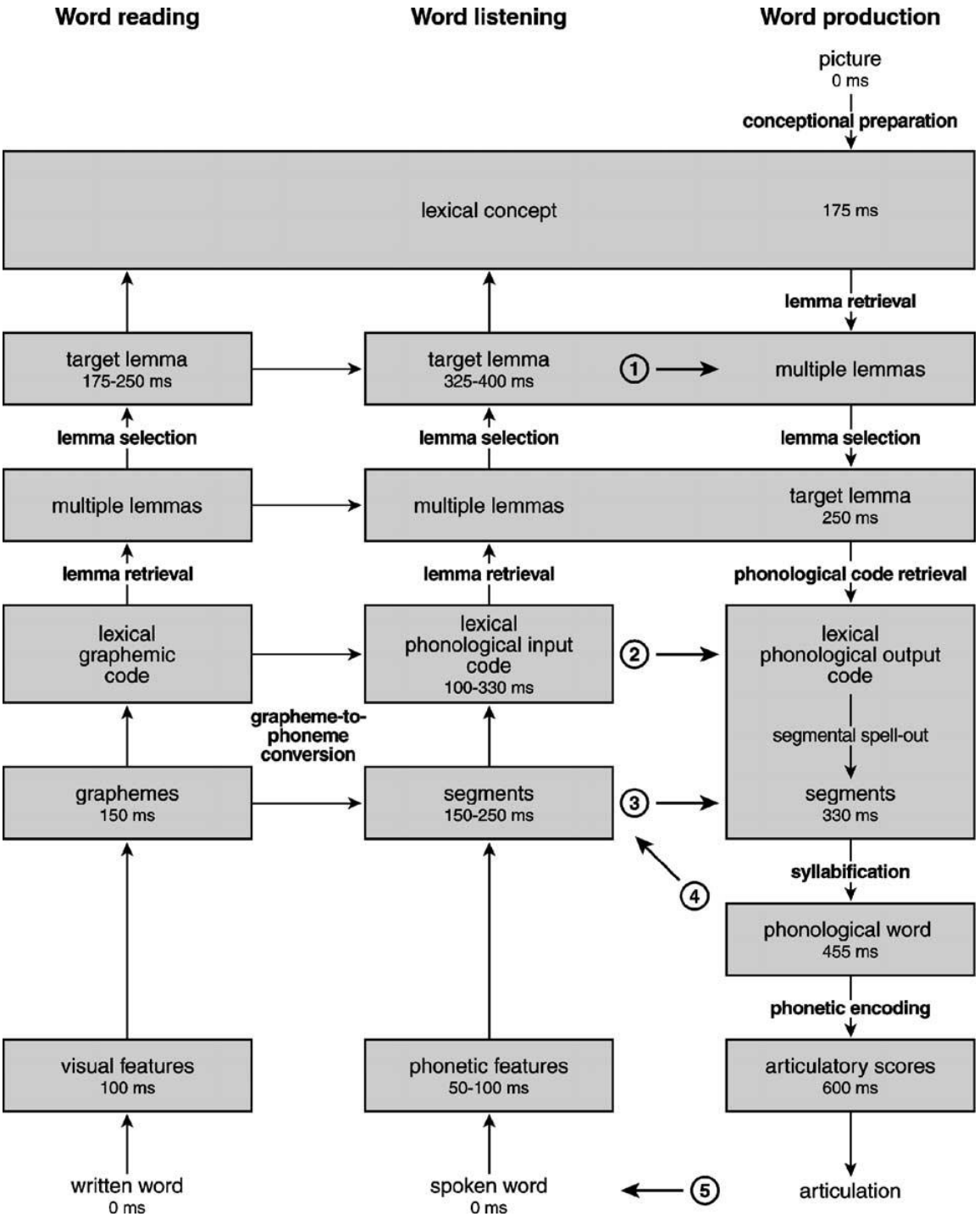

Figure 1.14 Network of processing components involved in the speech production and perception. Left column: assumed processing steps in word reading. Middle column: assumed processing steps in word listening. Right column: core processes of word production.

From *Indefrey and Levelt, 2004*.

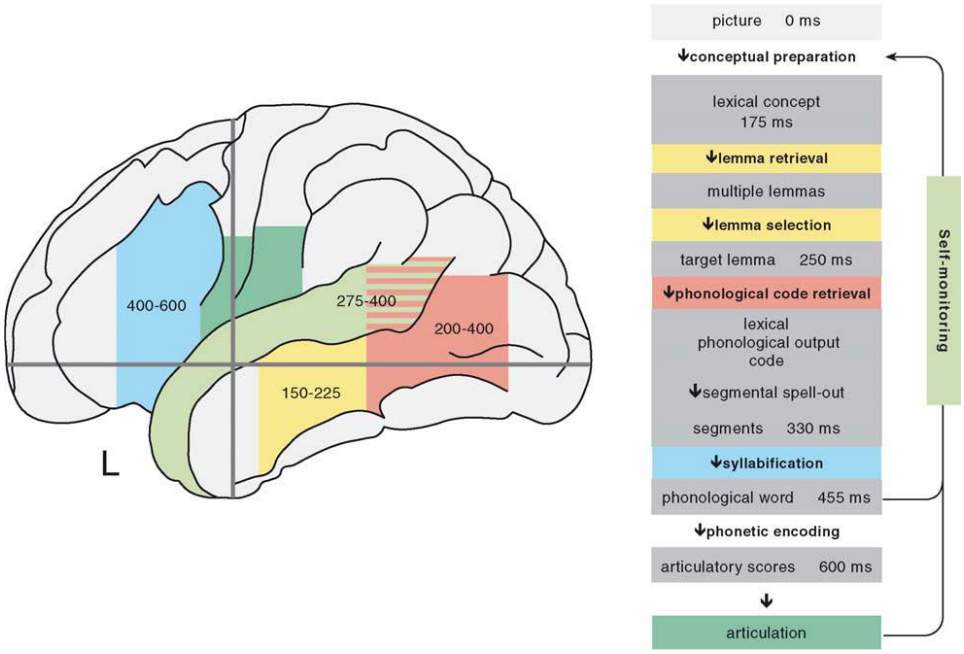

Figure 1.15 Time-reconstruction of human language in left hemisphere. Left column: schematic representation of meta-analysis results for word production. Identical colours indicate relations between regions and functional processing components (right column). The numbers indicate the time windows (in milliseconds) during which the regions are activated in picture naming. Right column: time course of picture naming.

From *Indefrey and Levelt, 2004*.

Motor execution

In a naming task, the motor execution has been estimated to occur within 600 ms following the picture presentation (**Fig. 1.14**). During motor execution, the brain areas activated, bilaterally, are the primary motor and sensory cortices, the ventral pre-motor cortices (**Fig. 1.15**), supplementary motor cortices, thalamus and cerebellum.

Subcortical fibres connecting language cortical areas

Historically the neuroimaging, lesion and electrophysiological studies allowed to study the cortical components of the language network but were not adequate to investigate the subcortical systems of fibres connecting the cortical areas. These connecting fibers were investigated with the *brain mapping* technique. This technique was developed in the first half of the XX century by Wilder Penfield (1891-1976), an American-born Canadian neurosurgeon. W. Penfield pioneered the technique of intra-operatory *brain mapping* by means of Direct Electrical Stimulation (DES) at present used during neurosurgical procedures for tumours removal or epilepsy treatment. During these procedures, the

patient undergoes general anaesthesia for the exposure and closure procedures, while during the central phase of surgery the general anaesthesia is switched to local anaesthesia and the patient is awakened. During the awake state, DES is delivered at the cortical and subcortical levels while patients perform a behavioural task and it is expected to interfere with the execution of the task only when the current is applied onto structures belonging to the neural circuit sub-serving the execution of that specific task. The analysis of the specific deficits in task performance induced by DES provides elements relevant to disclosing the role of the stimulated area in the brain network controlling that task.

As a testament to intuition of W. Penfield, the technique of *brain mapping* has been largely unchanged over time except for minor modifications in this above described technique, and can also be performed extra-operatively with implanted electrode arrays (Chang et al., 2015).

The clinical impact of the W. Penfield technique on prognosis, i.e. increased extent of tumour resection and decreased occurrence of deficits, was so dramatic that its use should be recommended in all the surgical procedures involving the language areas and other areas sub-serving the most important neural functions (Garrett et al., 2012).

More recently, W. Penfield's *brain mapping* technique was also applied to the subcortical structures, allowing the disclosure of the most relevant white matter tracts involved in the language function: the superior longitudinal *fasciculus* (SLF) and arcuate *fasciculus* (AF) in the dorsal stream and the inferior frontal-occipital *fasciculus* (IFOF) and the uncinate *fasciculus* (UF) in the ventral stream (Chang et al., 2015. **Fig. 1.16**).

Dorsal stream connections

The SLF (**Fig 1.16**) is composed of 4 major subcomponents: the SLF I, II, and III -connecting the frontal and parietal cortices-, and the SLF-tp subcomponent -connecting the temporal and parietal lobes-.

SLF I is not significantly involved in language processing and so will not be discussed. SLF II connects the dorsal pre-motor and pre-frontal cortices to the angular *gyrus* in the parietal lobe, whereas the operculo-opercular pathway known as the SLF III connects the ventral pre-frontal areas to the supramarginal *gyrus* in the parietal lobe. The SLF-tp runs in a posterior direction from the inferior parietal lobe to the posterior temporal lobe.

The stimulation of SLF II and III during language tasks induces dysarthria and other impairments in phono-articulatory processing, while SLF-tp is considered the language tract for sensory-motor integration of acoustic information.

The AF (**Fig 1.16**) connects the frontal-opercular cortical regions with the posterior temporal cortex. Despite it is considered the white matter tract connecting canonical Broca's and Wernicke's areas, the AF has been now shown to have also other targets. The frontal terminations include the *pars opercularis* of the Broca's area (BA44) as well as the ventral pre-motor cortex; the most relevant temporal terminations are the posterior superior and middle temporal *gyrus*; however, post-mortem cortex-sparing dissection techniques and tractography suggest that the AF may also extend caudally to the inferior temporal *gyrus*. The AF can be accurately and reliably identified intraoperatively based on the observation that its stimulation leads to phonological errors. Syntactical errors have also been ascribed to the AF based on tractography and subcortical stimulation studies (Chang et al., 2015).

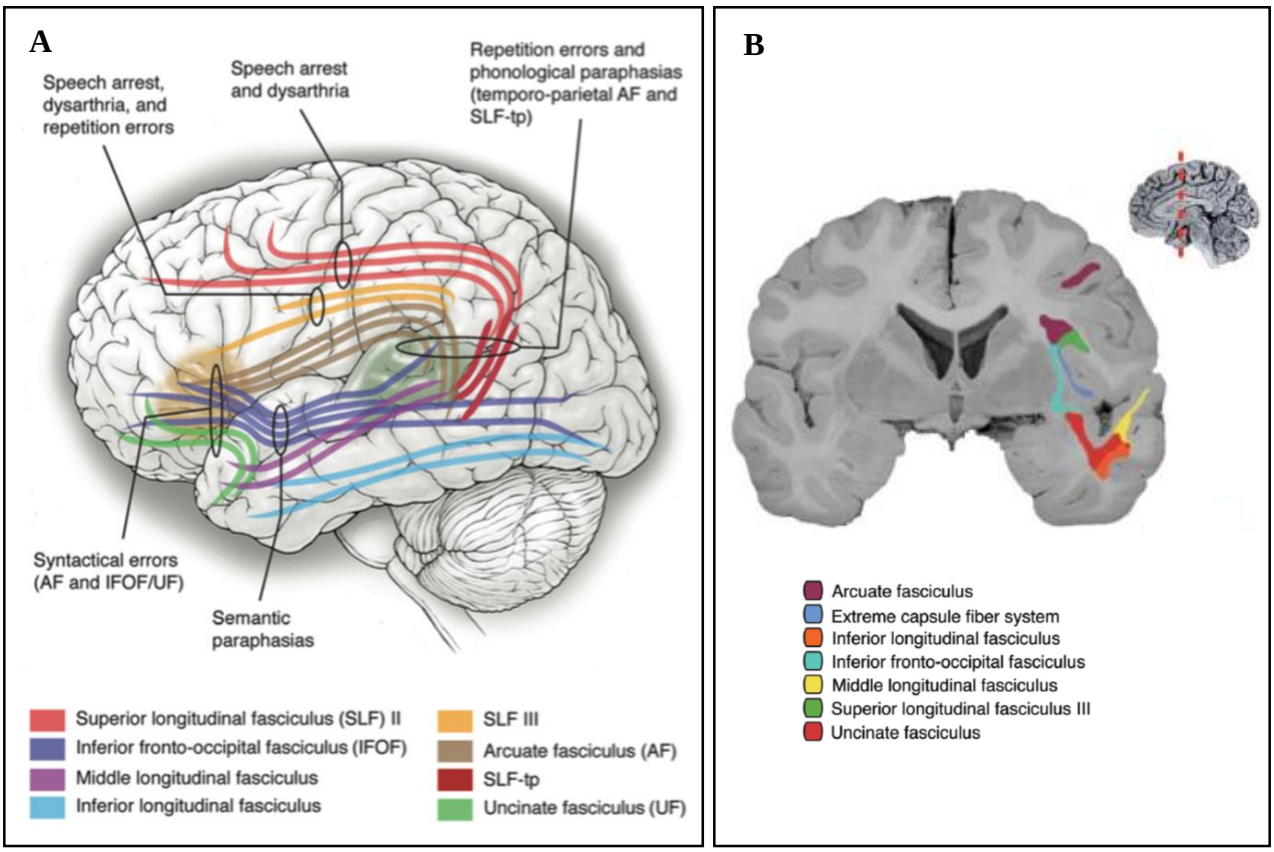

Figure 1.16 Schematic illustration of various subcortical tracts involved in language processing. Individual white matter tracts are colour coded (see legends). The subcallosal fasciculus and the frontal aslant tract (FAT) are not shown. In the panel A) are represented also the language errors induced by means of DES, when the stimulus is applied onto the fasciculus during speech tasks.

From *Chang et al., 2015 (A), Dick et al., 2014 (B).*

Ventral stream connections

The IFOF (**Fig 1.16**) is an anterior-posterior white matter bundle connecting the inferior frontal cortex and dorsolateral pre-frontal cortex to the posterior temporal and occipital lobes. Running through the

anterior floor of the external capsule, the IFOF sets medially in the temporal lobe and sends radiations to the middle and inferior temporal *gyri* as well as the occipital lobe. The direct electrical stimulation of this bundle elicits semantic paraphasias.

The UF (**Fig 1.16**), connecting the anterior temporal lobe to inferior frontal areas, is suggested to play a role in semantic function, although conflicting evidence challenge the exclusive effect of the resection of UF on language ability. Recent studies demonstrated a functional role of UF in the retrieval of proper names (Papagno et al., 2011) as well as in in decision-making processes (Olson et al., 2015).

Other connections

In addition to the *fasciculi* belonging to dorsal and ventral stream, other fibres are involved in language function such as the inferior longitudinal *fasciculus* (ILF), the middle longitudinal *fasciculus* (MLF), the subcallosal *fasciculus* and the frontal aslant tract (FAT).

Most of the ILF terminations emerge in the occipital-parietal region and in the ventral aspect of the temporal lobes (**Fig. 1.16**). In its posterior portion, the ILF fibres run vertically from the posterior lingual and fusiform *gyri* and *cuneus*. At the ventral aspect of the occipital-temporal junction, the ILF changes direction and runs horizontally in the white matter of the inferior temporal lobe, amygdala, and parahippocampal *gyrus*, to merge with the origin of the uncinate *fasciculus*. The posterior part of the ILF is involved in reading and face and object recognition, whereas both the anterior and posterior parts of the ILF are involved in the processing of names, reading, and spoken language. The intraoperative stimulation of the posterior ILF induce alexia (or acquired dyslexia), frequently associated to anomia or phonemic paraphasia when simultaneously stimulated with the posterior segment of the arcuate *fasciculus* (SLF-tp) (Kinoshita et al., 2016).

The MLF includes all long post Rolandic cortico-cortical association connections of the superior temporal *gyrus* and dorsal temporal pole with the parietal and occipital lobes (**Fig. 1.16**). Makris and colleagues (2016) suggested that this tract may be related to language, even if the intraoperative stimulation of MLF does not seem to generate any disorders (De Witt Hamer et al., 2011).

The subcallosal *fasciculus* (not shown in figures) runs between the caudate and the cingulate *gyrus* and/or SMA. When stimulated it shares similar features, i.e. a transient deficit in speech initiation with intact repetition of the well-known "SMA syndrome", following surgical lesions of the SMA proper (Chang et al., 2015).

The FAT, recently described (**Fig. 1.17**), connects pre-SMA and the most anterior SMA (face region) with the *pars opercularis* (or BA44) and the ventral pre-motor cortex (Vergani et al., 2014). Lesions of this tract has been associated with transient speech initiation disorders (Kinoshita et al., 2015) and

verbal fluency performance in people with primary progressive aphasia (Catani et al., 2013). Stimulation of FAT elicits, from anterior to posterior: speech inhibition (in only left hemisphere), both speech and motor inhibition, and motor inhibition.

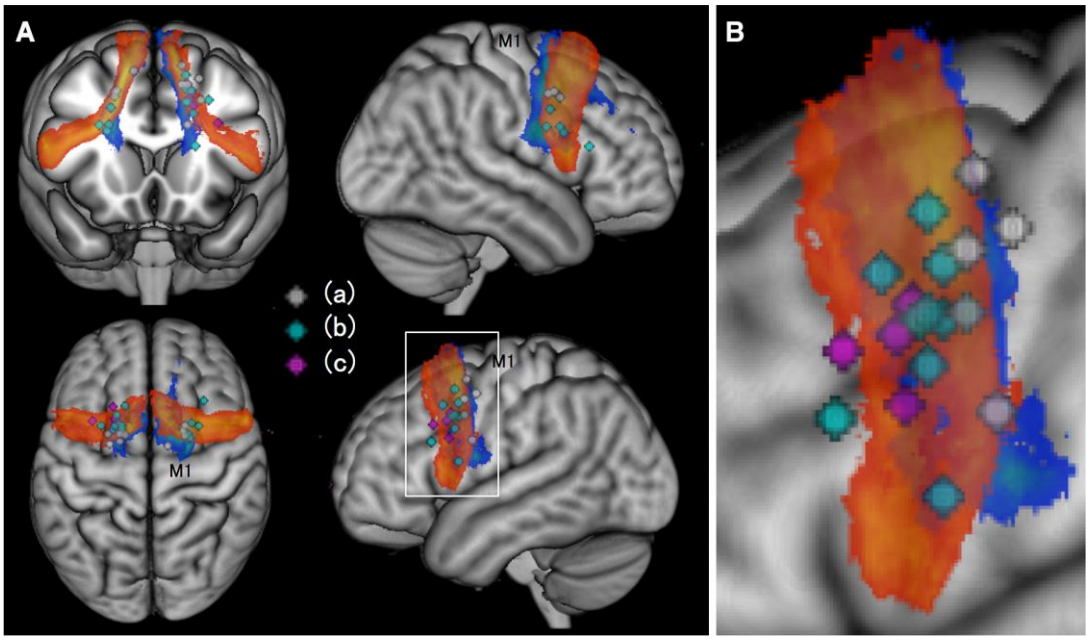

Figure 1.17 Three-dimensional overlap maps of intraoperative mapping and post-operative tractography of all patients. A) FAT (red-yellow) and frontal-striatal tract FST (blue-green) are overlaid with subcortical locations of sites eliciting inhibition of speech and/or motor actions during intraoperative electrical stimulation. A white line box in the sagittal image of left hemisphere is magnified in B) grey dots (a) motor inhibition; cyan dots (b) both speech and motor inhibition; violet dots (c) speech inhibition.

From *Kinoshita et al., 2015*.

1.3 THE SENSORY-MOTOR SYSTEM INVOLVED IN SPEECH PRODUCTION

According to the modern model of human language, this ability is sub-served by a complex system organized in three sub-systems:

- the syntactic rules and representations
- the sensory-motor interface
- the internal conceptual-intentional interface

The main aim of this PhD project (see the "Aim of PhD project" section) was to investigate "the cortical component of the sensory-motor system" underlying language, focusing on the characterization of the cortical areas involved in the motor control of speech. As described above, the sensory-motor system is composed of a complex cortical and subcortical architecture controlling the precise sequence of events involved in the production of sounds, at a rate up to six to nine syllables per second or 20 to 30 phonetic segments per second (Kent, 2000), occurring once the linguistic form of a word or a phrase has been computed. To operate such a complex function, the system finely coordinates the phono-articulatory apparatus (Fink, 1986; Berwick et al., 2013), involving about 100 muscles (Kent, 2000), by means of corticobulbar tract.

Cortical motor areas involved in speech production

Inside of the sensory-motor system, the Primary Motor (M1), ventral Pre-Motor (vPM), Supplementary Motor (SMA), Broca's and Insula areas represent the five frontal areas mostly involved in motor control of speech production.

The cortical areas belonging to sensory-motor system are represented in **Figure 1.18**. For simplicity only the left hemisphere, the *dominant* hemisphere for the language system (see above), is represented, even though it is well known the bilateral involvement of M1, vPM, SMA and Insula cortex in speech production (Indefrey and Levelt, 2004; AbdulSabur et al., 2014), while Broca's area is localized only in left hemisphere. In figure:

- M1 corresponds to most of the pre-central *gyrus* and to Brodmann's area 4 (BA4);
- vPM is the most anterior portion of the pre-central *gyrus*, posterior to the pre-central sulcus and corresponds to the ventral portion of BA6;
- SMA is located anterior to M1, on medial wall of the frontal lobe, superior to the cingulate sulcus and corresponds to the medial portion of BA6;

- Broca's area is localized in the posterior portion of inferior frontal *gyrus*, anterior to pre-central sulcus and to vPM, and corresponds to BA44-45;
- the insula cortex ("the fifth or hidden lobe"), it is located deep to the temporal, parietal, frontal lobes, under the Sylvian scissure, and corresponds to BA13.

Focus of my PhD project were in particular three out of five cortical areas: M1, vPM and Broca's area, thus, a major characterization of these areas will be reported.

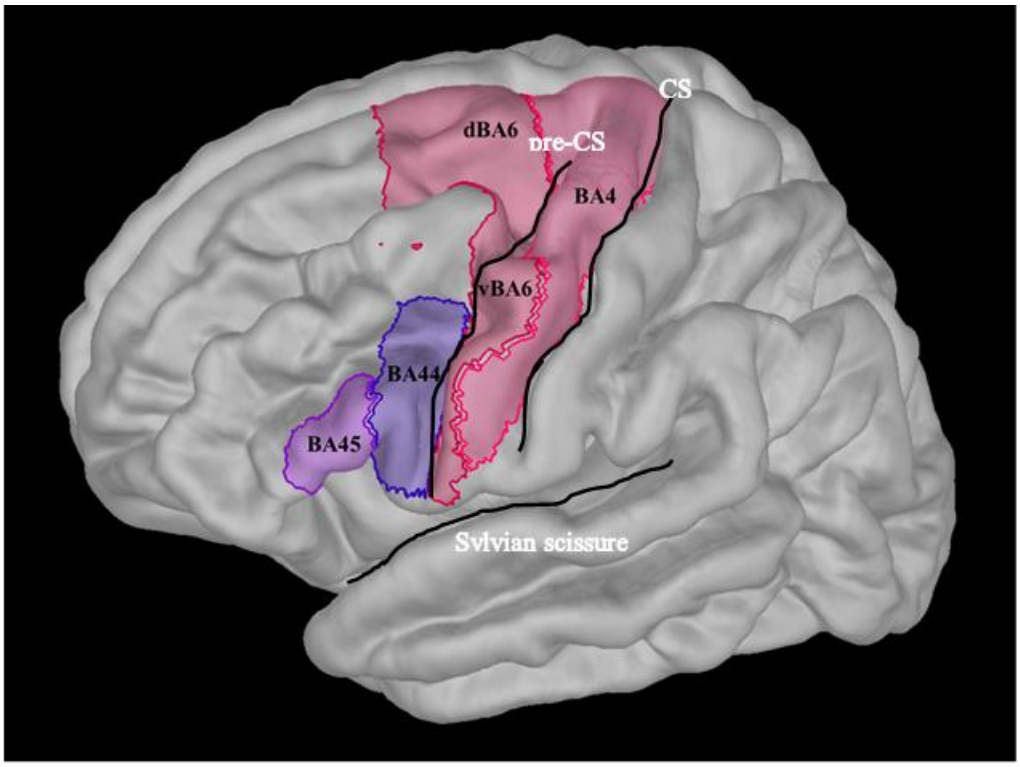

Fig. 1.18 Representation of the main cortical structures responsible of the motor direct coordination of the phono-articulators. These areas are found in the frontal lobe: primary motor cortex (M1) is indicated as Brodmann's area 4 (BA4), the ventral pre-motor cortex (vPM) as the ventral portion of BA6 (vBA6), the supplementary motor area (SMA) as the dorsal-mesial part of BA6 (dBA6), Broca's area as BA44 and BA45. The insula cortex is, instead, located deep in Sylvian scissure (not shown).

CS = central sulcus; pre-CS = pre-central sulcus

Cyto-architectonic layers: M1, vPM and Broca's area

In the early 20th century, the two neurologists Alfred Walter Campbell (1868-1937) and Korbinian Brodmann (1868-1918) subdivided the human cerebral cortex in a six horizontal layers architecture (**Fig. 1.19**) based on localization of a cells group called "pyramidal neurons". These are highly polarized neurons, with their major axis perpendicular to the pial surface of the cortex. Their cell body is triangular, with a large number of dendrites that emanate from the apex and form the base of

the cell body. The span of dendrites is variable and consequently their function. The pyramidal cells located in Layer IV (see below), called large pyramidal cell, with an expanse dendritic tree, project their axons toward the subcortical white matter, sending collateral branches targeting other cortical domains proximal to the cell of origin. Pyramidal cells with circumscribed dendritic trees allow the vertical communication among layers, forming small microcircuits called cortical "columns".

In **Figure 1.19** are represented the six cortical layers, from the pial surface (above) to white matter (below):

- the Layer I, or molecular layer, contains few neurons and consists mainly of extensions of dendrites of pyramidal neurons and horizontally oriented axons, as well as glial cells. In addition, some spiny stellate cells can be found;
- the Layer II, or external granular layer, contains small pyramidal neurons and numerous stellate neurons;
- the Layer III, or external pyramidal layer, contains predominantly small and medium-size pyramidal neurons, as well as non-pyramidal neurons with vertically oriented axons;

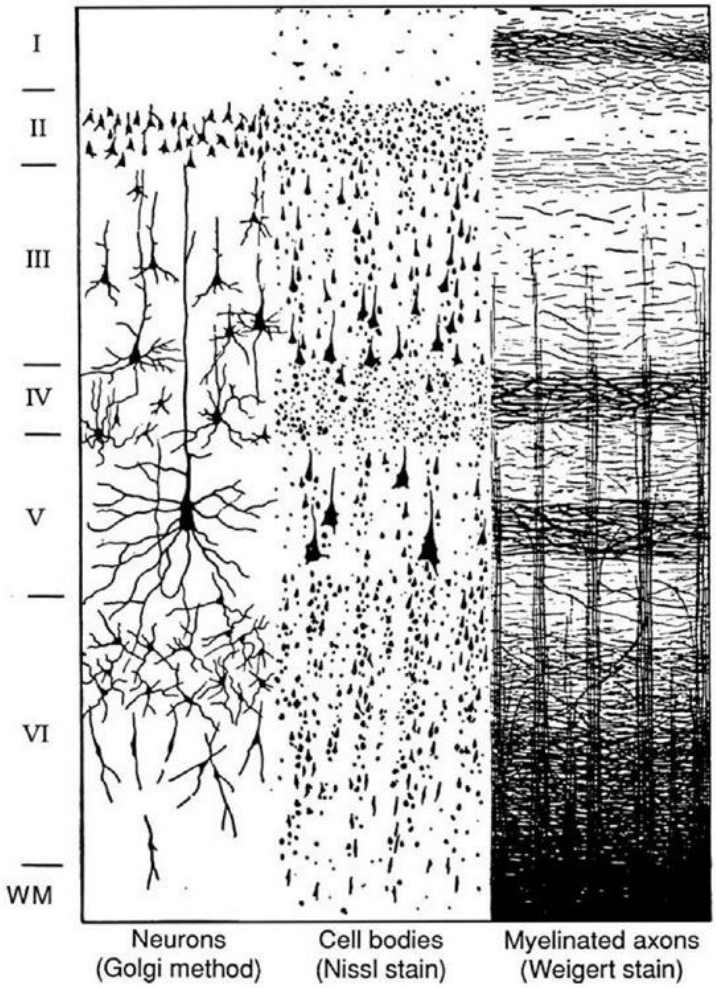

Fig. 1.19 The figure represents a magnification of the cortical architecture of the cerebral cortex (thickness between 2 and 4 millimetres), obtained by three different histological staining: Golgi method to detect the neurons, Nissl stain to detect cell bodies and Weigert stain to detect the myelin. At left, the six cortical layers that compose the cortex are indicated with roman number, from I (under pial surface) to VI (before of the white matter, WM).

- the Layer IV, or internal granular layer, contains different types of stellate and pyramidal neurons;
- the Layer V, or internal pyramidal layer, contains large pyramidal neurons which give rise to axons leaving the cortex and running down to subcortical structures;
- the Layer VI, or polymorphic or multiform layer, contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons.

The cyto-architectonic subdivision of the cerebral cortex described by A.W. Campbell and K. Brodmann varies among the cortex, therefore based on histological difference the cortex has been partitioned in a large number of different areas. Among the language areas object of our investigation, M1/BA4 lacks of the distinct internal granule cell Layer (IV), so it is called "agranular cortex", and it contains the giant pyramidal cells, or Betz cells, in Layer V. These two cyto-architectonics features allow to discriminate M1/BA4 from adjacent cortices, for example from the pre-motor cortex/BA6. Differently to M1/BA4, BA6 contains less and smaller giant pyramidal cells in Layer V and in addition, the Layer IV is "dysgranular" (Book: "Principles of Neural Science", Kandel, Schwartz, Jessell, Siegelbaum, Hudspeth, the 5th edition, 2014). These features distinguish, in turn, the pre-motor/BA6 cortex from its neighbouring rostro-ventral region localized in the inferior frontal *gyrus*, i.e. the Broca's area or BA44-45. In particular, BA44 is discriminated on the basis of conspicuously large pyramidal cells in deep Layer III and V, and by a barely recognizable dysgranular Layer IV, which is invaded to different degrees by Layer III and V pyramidal cells. BA45 differs essentially from BA44 for the presence of a clearly visible Layer IV (**Fig. 1.20**)**,** more conspicuous than the analogue layer in BA44, but much less visible than Layer 4 clearly observed in the rostral adjoining pre-frontal cortex, e.g. in BA10 or 46 (Amunts et al., 1999).

A recent subdivision based on the distribution of receptors (panel B in **Fig. 1.20**, Amunts et al., 2010, 2012) subdivides BA44 and BA45: the ventral and dorsal part of BA44 -vBA44 and dBA44 respectively- differ for the expression of muscarinic M_2, AMPA, and alpha1 receptors, while BA45, subdivided in anterior and posterior portion -aBA45 and pBA45 respectively-, based on the different expression of M_1, AMPA and alpha1 receptors. The different density of kainate, $GABA_A$ and alpha1 receptors allow to distinguish Broca's area (BA44) from pre-motor/BA6 cortex.

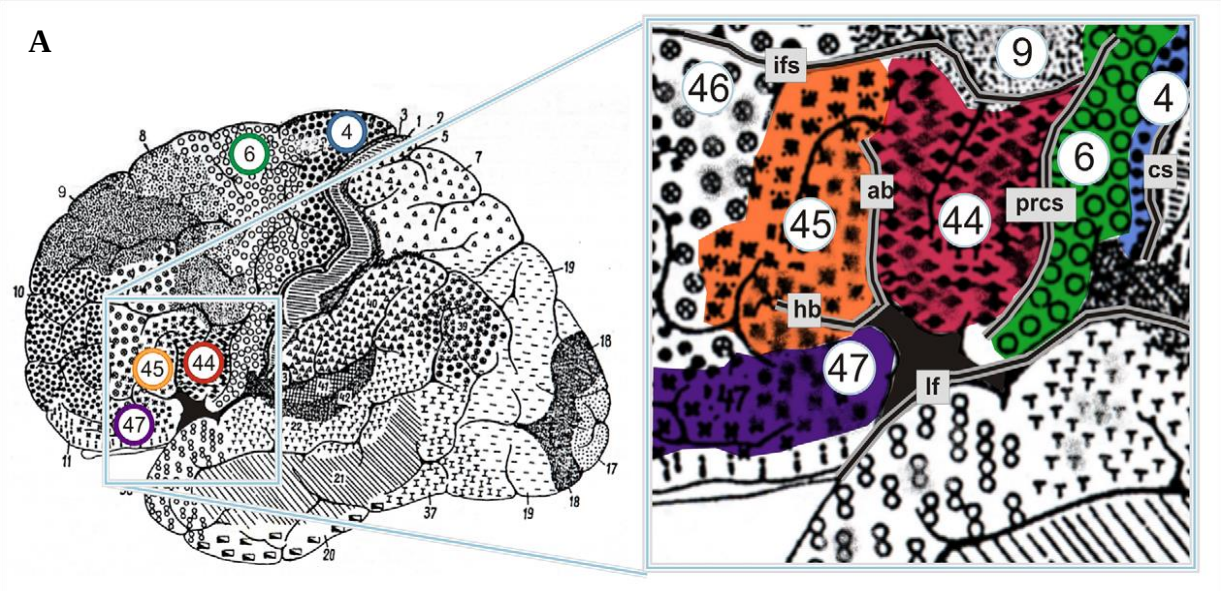

Fig. 1.20 Cyto-architectonic maps of Broca's area. The panel **A** represents the map of the lateral surface of a human cortex adapted from Brodmann. The region of interest contains BA44 and BA45 as well as parts of the neighbouring BA4, BA6, and BA47. "ab" = ascending branch of the lateral fissure; "cs" = central sulcus; "hb" = horizontal branch of the lateral fissure; "ifs" = inferior frontal sulcus; "lf" = lateral fissure; "prcs" = pre-central sulcus. The panel **B** represents the map based on the distribution of receptors of neurotransmitters and shows a complex segregation of the inferior frontal *gyrus* and adjacent cortices. In particular, it shows the presence of a series of new areas in the inferior frontal sulcus (ifs) labelled as areas ifs1, ifs2, ifj1 and ifj2, as well in the frontal operculum ('op'-areas).

From *Amunts et al., 1999, 2013, modified.*

Cortical phono-articulatory muscle representations

The direct electrical stimulation studies showed that the motor areas contain a "motor map" of the contralateral body muscles and that the integrity of this motor map is mandatory for voluntary movement of the corresponding body parts. Being this PhD thesis focused on the cortical areas

involved in motor control of speech, it is important to highlight the cortical regions controlling to phono-articulatory apparatus distinguishing them from those controlling other body districts.

The evidence available in humans at present on this topic were obtained by recording brain activity with indirect methods (fMRI, MEG, EEG, Indefrey 2011; Flinker et al., 2015) during language tasks, by recording responses or interference effects induced by stimulation (direct electrical stimulation, DES, and transcranial magnetic stimulation, TMS) of different cerebral areas during language tasks (Tate et al., 2014; Chang et al., 2016) and/or in resting state condition (Cerri et al., 2015), and from post-mortem micro-dissection studies (Kuypers, 1958b). However, a somatotopic map of the phono-articulatory district is not, at present, fully disclosed. The most precise somatotopic description arises from recording studies (intracortical microstimulation, Dum and Strick, 2005) in monkey. In this setting, though the somatotopic information is related to localization of the representation of oro-facial muscles presumably involved in motor skills not related to language, since the monkeys do not speak.

M1/BA4

The primary motor cortex (M1/BA4, **Fig. 1.18**) is organized somatotopically in the pre-central *gyrus*, in a dorsal-ventral fashion (**Fig. 1.21**): the muscles controlling the lower limb lies on the mesial hemisphere, approximately to the median line; ventrally, on the convexity and near the Sylvian scissure, the muscles of the face, including the tongue, lips and larynx, are represented; the upper limb and axial muscles lie in between these two areas. The proximal and axial muscles are represented on the exposed surface of the cortex, while the distal muscles, as the tongue and lips, are represented in the bank of the central sulcus. The representation of each muscle district is proportional to the innervation density of peripheral muscles, i.e. by the number of motor units that control the muscles of the district. The segments involved in the execution of fine movements (for example fingers, lips, tongue) require a refined-control of force recruitment and are served by a high number of motor units. The graphical representation of this peculiar organization is the famous "*motor homunculus*" (left panel in **Fig. 1.21**).

The introduction of the intracortical microstimulation (ICMS) technique allowed to stimulate the cortex in more focally and highlighted the columnar organization of M1. The activation of a muscle (or muscles acting on the same joint) could be induced by stimulating separated cortical sites (or more precisely "columns"), often not contiguous, in the bank, suggesting that the cortex hosts multiple “columns” of neurons controlling a muscle acting on a given articulation and that these columns are mixed with columns controlling muscles acting on other joints. It follows that within a macroscopic region controlling a body segment, there is a complex set of “columns” arranged in such a way as to

group the representations of different muscles involved in specific movement. In this model, the organization of a multi-joint complex movement requires of local connections within neurons of "columns" close to each other, in which the different muscles involved in the movement are represented (Book: "Principles of Neural Science", Kandel, Schwartz, Jessell, Siegelbaum, Hudspeth, the 5th edition, 2014).

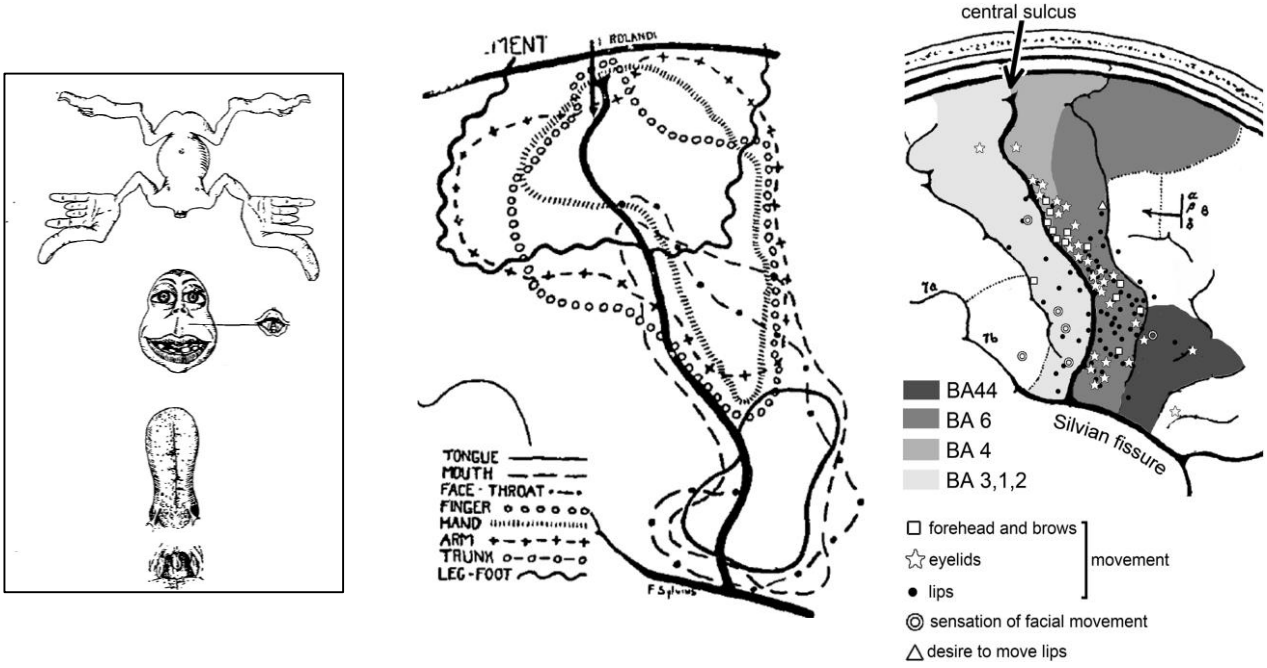

Figure 1.21 *Motor homunculus*. At left: Visualization of the order and comparative size of the parts of the body as they appear from above down upon the Rolandic cortex. The larynx represents the vocalization, the pharynx swallowing. The comparatively large size of thumb, lips and tongue indicate that these members occupy comparatively long vertical segments of the Rolandic cortex. In the middle: the lines enclose the areas within which responses were obtained for each subdivision of the body (original representation of Penfield and Boldrey). At right: the points represent where movements of different facial parts were evoked by electrical stimulation. BA = Brodmann's area.

From *Penfield and Boldrey, 1937* and *Cattaneo and Pavesi*, 2014.

Phono-articulatory representation in M1. The portion of M1/BA4 that controls the facial muscles (McGuinness et al., 1980; Sessle and Wiesendanger, 1982), involved in phono-articulation, is the most ventral (**Figure 1.21,** Kuypers, 1958b; Morecraft et al., 2014). This region, as well as that of monkey and chimpanzee, contains the cortical neurons that have direct connections with the pre-motor interneurons and with the motoneurons in the brainstem (in pontine and medullary lateral tegmental field, Kuypers, 1958a; Holstege and Subramanian, 2015) which, in turn, innervate, in human, the principle phono-articulatory muscles: face, mouth, lip, larynx, pharynx, and tongue muscles. The cortical representation of facial muscles in M1, obtained with DES, shows a dorsal-ventral fashion: face and throat, mouth and lip, larynx and pharynx, and tongue muscles (**Fig. 1.21**

and **1.23** in the middle and at left. Penfield and Boldrey, 1957; Simonyan et al., 2011). The same organization has been demonstrated with transcranial magnetic stimulation (TMS, Cattaneo and Pavesi, 2014). In these studies has been added that perioral muscles and *orbicularis oris* –important phono-articulatory muscles- seem to be more extensively represented than *orbicularis oculi* and *frontalis* muscles –not involved in phono-articulatory gestures. All oro-facial muscles seem to receive a bilaterally innervation although with predominant control by the contralateral side. This is the case of *lip depressors* (Meyer et al., 1994; Rödel et al., 1999), of muscles active in protusion of lips (Yildiz et al., 2004; Triggs et al., 2005), of the *buccinator* muscle (Urban et al., 1997, 2001), the *nasalis* muscle (Dubach et al., 2004; Fischer et al., 2005) and of the *depressor anguli oris* (Pilurzi et al., 2013). The cortical representation of the *orbicularis oris* muscle seems, instead, to be more symmetrical (Fischer et al., 2005).

A TMS study (Liscic et al., 1998) suggests monosynaptic connections connecting cortical neurons and motoneurons driving oro-facial muscles as shown in monkey's studies.

Interestingly no clear border (**Fig. 1.21**) separates, in Penfield's representation, M1/BA4 from the rostral portion of pre-motor cortex (BA6) –at present better investigated (see **Fig. 1.18)**-. This observation could be due to the fact that the rostral cyto-architectonic border of M1/BA4 in the lateral part of the Rolandic sulcus is blurred: it recedes caudally and eventually disappears in the rostral bank of the central sulcus (Geyer et al., 2000). As a result, in humans it could be challenging to distinguish M1/BA4 facial representation from that of BA6.

vPM/vBA6

Different studies using different approaches (Indefrey and Levelt, 2004; Brown et al, 2008; Tate et al., 2014; Tomasino et al., 2014; Chang et al., 2015; New et al., 2015) demonstrated the involvement of the ventral pre-motor cortex (vPM/vBA6, **Fig. 1.18)** in motor control of speech. However, in humans, due to the huge confusion about the functional-anatomical border between M1 and vPM, in many studies these two areas were considered as a unique area in motor control of speech. Brown and colleagues (2008), investigating M1/BA4 and vPM/vBA6 activation during oral tasks execution (vocalization, glottal stops, lip protrusion, vertical tongue movement), contributed to reconstruct a map that collectively addresses somatotopy in both areas, suggesting a common motor region controlling adduction/abduction and tensing/relaxing of the vocal folds, called *larynx/phonation area* (**Fig. 1.22** indicated in orange) localized adjacent to the lip area (**Fig. 1.22** indicated in light blue) and more dorsal to the tongue area (**Fig. 1.22** indicated in green).

Simonyan and Horwitz (2011) suggested that the larynx area is surrounded by the representations of the tongue, lip, and masticatory muscles and it is considered to be part of M1/BA4, although cyto-architectonically it corresponds to vBA6, also in not-human primate (**Fig. 1.23**).

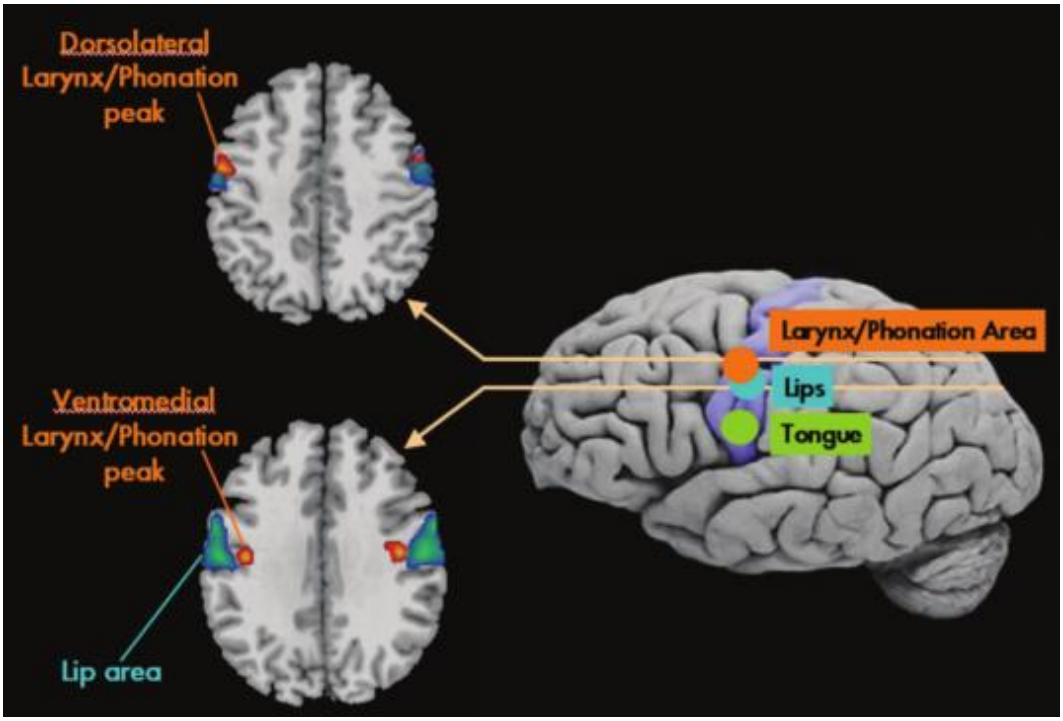

Fig. 1.22 The right side shows a human brain with the approximate locations of the somatotopic representations for the lips, tongue and larynx.

From *Brown et al., 2008*

Non-human primate. The ventral-lateral PM in non-human primate is known as F5. F5 of *Macaca* and *Aotus Trivirgatus* contains mainly the mouth-face-neck representations, mixed with the eyelid movements representation (Gentilucci et al., 1988; Huang et al., 1988; Rizzolatti et al., 1988; Godschalk et al., 1995; Preuss et al., 1996; Maranesi et al., 2012). The mouth component is located more ventrally in comparison with movements involving the upper face and neck, located in the mid-portion of F5 (Graziano, 2006; Graziano and Aflalo, 2007), confirming a muscle somatotopy. However, F5 is demonstrated to have a crucial role not only in the control of goal-directed actions performed with mouth (Ferrari et al., 2003; Coudè et al., 2011), but also performed with the hand (Jeannerod et al., 1995; Umiltà et al., 2008; Bonini et al., 2011; Rizzolatti et al., 2014) and these two body representations are considerable overlapped (Maranesi et al., 2012). F5 is distinguished from the more dorsal sector of the pre-motor cortex (F2), uniquely involved in control of arm movements for reach and grasp (Johnson et al., 1996; Wise et al., 1997; Raos et al., 2004; Hoshi and Tanji 2007). Other studies have also shown consistent differences between F5 and the posterior border, M1,

probably reflecting the different hierarchical role of these two areas in the control of spinal motoneurons (see below). These studies have suggested that the motor output from F5 is mostly mediated through cortico-cortical connections with M1, through the corticospinal projections (Cerri et al., 2003; Shimazu et al., 2004; Schmidlin et al., 2008; Prabhu et al., 2009), while the motor output from M1 is mediated by the corticospinal projections. These monkey studies distinguished F5 from neighbouring cortical areas, both at functional and anatomical level.

Human primate. In human primate, instead, there is a confusion about the functional-anatomical border between M1 and vPM, even if a functional subdivision of vPM/vBA6 from its dorsal sector

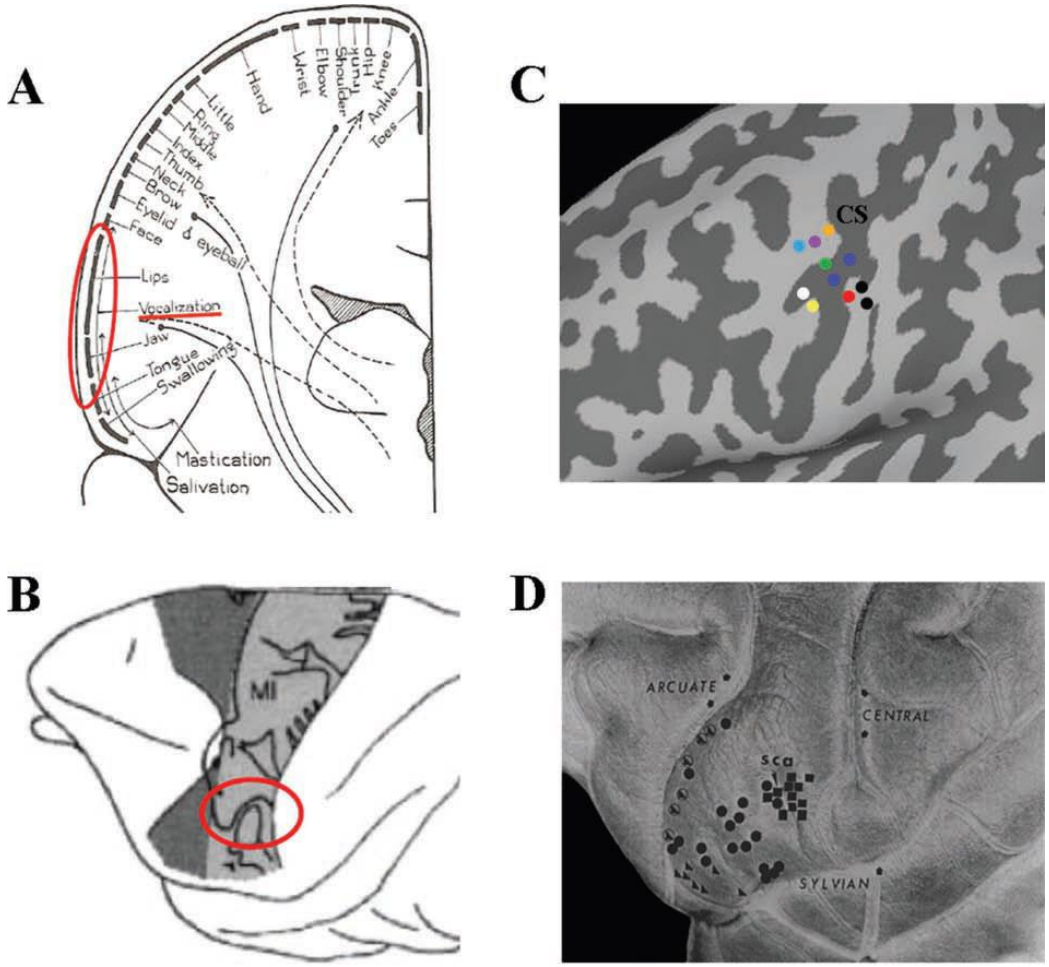

Fig. 1.23 Laryngeal motor cortical representation. **A.** Schematic views of body representation within of M1 in humans ("motor homunculus" according to Penfield and Boldrey 1937) and **B.** in the *rhesus* monkey ("motor simiculus" according to Woolsey et al., 1952). **C.** The laryngeal motor cortical region in humans as defined in neuroimaging studies. The coloured circles represent the reported peaks of activation in different studies of syllable production: orange, Bohland and Guenther (2006); purple, Olthoff and others (2008); light blue, Terumitsu and others (2006); green, Loucks and others (2007); blue, Brown and others (2008); red, Wilson and others (2004); black, Simonyan and others (2009); white, Riecker and others (2008); and yellow, Peeva and others (2010). CS = central sulcus. **D.** The laryngeal motor cortical region in the *rhesus* monkey. Topographical representation of the laryngeal muscles: cricothyroid = right-angled triangle; thyroarytenoid = circle; combination of the cricothyroid and thyroarytenoid = encircled right-angled triangle; extrinsic laryngeal muscles = square; sca = sulcus sub-centralis anterior.

From *Simonyan and Horwitz, 2011.*

(dPM/dBA6) emerges in fMRI studies: both vPM and dPM are activated by upper limb motor tasks (Binkofski et al., 1999; Ehrsson et al., 2000, 2001; Kantak et al., 2012), while oro-facial motor tasks (including biting and speech-related movements) activate only vPM, suggesting that, in analogy with the monkey, this subdivision hosts both hand and oro-facial representations.

The literature regarding the role of vPM in motor control of speech deserves a detailed description. The research techniques mostly adopted to investigate the role of vPM in speech fall in three categories: 1) imaging studies, 2) studies on lesions in stroke patients and in patients affected by degenerative pathologies and 3) intraoperative studies conducted by means of *brain mapping* technique.

1) Imaging studies. In meta-analysis studies (Indefrey and Levelt, 2004; Chen and Desmond, 2005a,b; Bohland and Guenther, 2006; Peeva et al., 2010; Indefrey, 2011), which considered production and reading of words, pseudo-words and syllables, vPM is activated during speech articulation.

2) Lesions studies. A paradigmatic disorder clearly affecting speech production is the so-called Apraxia of Speech (AoS). The AoS is defined as a disorder of the phono-articulatory function. Apraxia is a neurological term referring to a complex motor deficit, which cannot be strictly attributed to pyramidal, extrapyramidal, cerebellar or sensory lesions (Kasper et al, Harrisons' Principles of Internal Medicine, 19th edition). Despite the different definitions that historically have been proposed for the term AoS, it was always reported related to a functional impairment of speech in absence of a reduced strength (or tone) of muscles of the speech articulators (lips, tongue, jaw and palate) or laryngeal muscles (Hillis et al., 2004; Ackermann and Riecker 2010). In 1996, Dronkers defined AoS as "a disorder in the motor program driving the speech laryngeal and phono-articulatory muscles to produce the correct sounds composing the words in the proper sequence with the appropriate timing", based on the observation that patients affected by AoS made inconsistent articulatory errors approximating the target word, showed articulatory groping and often had an associated disruption in prosody and rate of speech. In 1999, AoS was reported as a neurological disorder characterized by an impaired ability to shape the vocal tract for a particular speech sound (spatial incoordination) and, to rapidly and accurately reshape the vocal-tract shape for the next speech sound (temporal incoordination) (Wise et al., 1999). In 2004, Hillis and colleagues added that AoS was characterized by distortions of consonants and vowels that might be perceived as sound substitutions and mis-assignments of stress. Furthermore, patients, affected by AoS, were aware of what they would like to say and of their errors. A detailed analysis of the phono-articulatory disorders in AoS patients showed that it was characterized by an increased morphemes durations of the words, segmentation of sounds

and syllables, speech sound distortions and dysprosody (Maas et al., 2008). Klapp in 2003 proposed the so called “INT/SEQ model” of speech production postulating that speech is produced by the correct sequencing (SEQ) of elementary units of motor actions (INT) and AoS was suggested to be a specific disorder of the INT phase of speech production (Maas et al., 2008). Interestingly, the clinical history of patients affected by AoS ranges from brief episodes of speech deficits to persistent severe abnormalities (Ackerman and Riecker, 2010). By combining all the evidence related to this disorder, Hickok and colleagues (2014) reported that the “errors” resulted by substitutions, deletions, transpositions, and insertions of speech sounds in words to be pronounced, the probability of errors increasing with increased length and complexity of utterances. In 2015, Ziegler and Aichert reported that the errors in motor programming were more likely to occur during consonantic sounds than in vocalic sounds production and that some consonantic gestures, as fricative or lateral consonants, were more sensitive to apraxic impairment than the complete oral closure type of constriction occurring in plosives and nasals. Furthermore, errors in pre-vocalic consonantic gestures are more frequent than errors in the post-vocalic consonantic gestures. However, the occurrence of an error is not exclusively related to the phoneme, which is incorrectly articulated, but the whole structure of the word contributes to the error itself and, consequently the speech of the patients is dysfluent and halting, with many false starts, restarts, and self-corrections.

Conclusively, the AoS seems to be a speech disorder associated with inefficiencies in the translation of speech sounds (phonemes) into proper phono-articulatory gestures, resulting in incorrect timing (prolongation of speech sounds/segments and between sound or segment gaps), distorted sounds, consistency in error type, and abnormal prosody (de-stressing of typically stressed syllables and sounds) (New et al., 2015).

This disorder is extremely interesting in that it clearly affects the phono-articulatory motor program and thus, by identifying the precise cortical/subcortical site of the lesion resulting in AoS, it might be possible to add new elements on the cortical control of phono-articulation. The analysis of patients affected by stroke, in the left hemisphere, showed that patients affected by AoS commonly displayed lesions of the anterior portion of the insular lobe (Dronkers, 1996), which was actually demonstrated to be possibly due to an artefact associated to the susceptibility of the anterior insula to infarcts of the Middle Cerebral Artery (MCA) (see the MCA localization in **Fig. 1.24**) which actually damages other regions (Hillis et al., 2004). Consequently, all the brain areas supplied by the MCA could to be responsible of AoS in these patients, and, among those also the Broca's area.

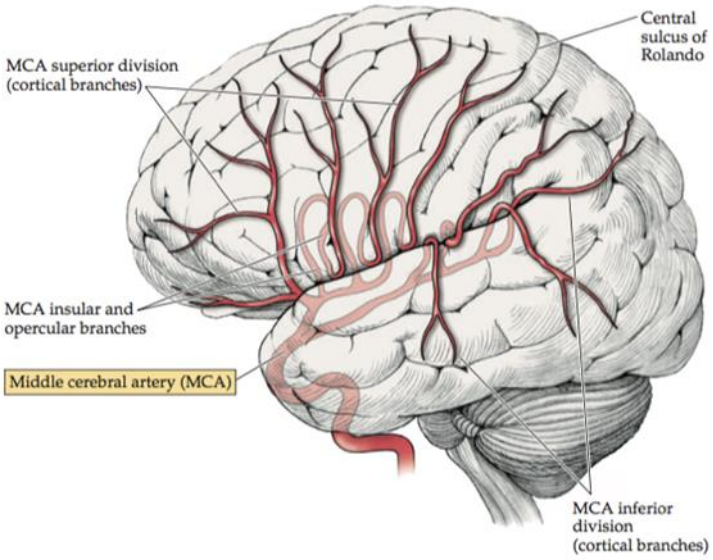

Fig. 1.24 Schematic representation of middle cerebral artery (MCA). MCA gives rise to inferior and superior divisions after the parting in the Silvian fissure.

A very refined study investigating stroke patients with pure AoS (excluding all patients with other deficits affecting speech) demonstrated a clear lesion affecting the left pre-motor cortices (Graff-Rafford et al., 2014). These data were supported by studies on neurodegenerative disorders affecting the same areas (Josephs et al., 2006). Interesting was the case (Fox et al., 2001) of patient with a focal stroke in the pre-central *gyrus* involving the motor and pre-motor cortices, presenting acute inability to speak, intact comprehension of both oral and written language, ability to communicate using a pictorial board and intact ability to write, only mild weakness of the right hemi face but spared hemi soma. As a permanent deficit, the patient was affected by AoS.

Altogether, these studies suggest that in patients with AoS the brain areas possibly significantly involved in the genesis of the deficit are M1/BA4 and vPM/vBA6. With regard to the latter, bilateral lesions of pre-motor cortex is critically associated with AoS and particularly the reduced connectivity between the left and right pre-motor cortices is correlated with severity of AoS and the decreased connectivity between the left pre-motor cortex and the anterior portion of the right insula is more often associated with non-verbal oro-facial apraxia severity (New et al., 2015). All data converge on the hypothesis that only pre-motor cortex lesions (vPM) are strictly associated to AoS and that, consequently, vPM has a role in speech motor programming.

3) Intraoperative studies. As reported previously, the direct electrical stimulation (DES), delivered on brain areas belonging to language circuit during language task performance, induces different interferences/effects. Different effects are routinely observed when DES is applied on the areas of the frontal lobe involved in speech production: 1) articulatory deficits, recently reported as dysarthria, 2) speech arrest/anarthria and 3) vocalizations. These different interference-effects do not

permit to distinguish vPM from the neighbouring speech areas (M1 and Broca's area). In all the intraoperatory studies considered so far, the speech production was evaluated during procedure by auditory and visual inspection of vocal output and of motor contraction of facial muscles.

Dysarthria is described as a speech impairment (Tate et al., 2014) or arrest (Petrovich et al., 2005) due altered mouth or pharyngeal muscles contraction (Petrovich et al., 2005; Deletis et al, 2014; Tate et al., 2014) leading in a dysphonic (Petrovich et al., 2005; Deletis et al, 2014; Tate et al., 2014) or aphonic (Deletis et al., 2014) speech. This interference-effect is reported by neurosurgical literature as a consequence of both vPM (Duffau et al., 2005; Petrovich et al., 2005) and M1 (Deletis et al., 2014; Mandonnet et al, 2016) stimulation, therefore it is difficult to distinguish, based on this effect during procedure, between vPM and M1.

Anarthria or Speech arrest are considered synonyms (Chang et al., 2011; Tate et al, 2014; Mandonnet et al, 2016) and are described as "the complete interruption of ongoing speech in absence of oro-facial movements and vocal output" (Petrovich et al., 2005; Sanai et al, 2008; Chang et al, 2011, 2015, 2016; Matsuda et al., 2014; Tate et al., 2014; Mandonnet et al., 2016). Differently from dysarthria, anarthria/speech arrest is characterized by the absence of a motor/muscle involvement and it is observed when DES is applied both on vPM and Broca's area. Again, based on this effect, it is not clear, during procedure, where the functional border between vPM and Broca's area is located.

Vocalization is described as "an expiratory vowel sound, in which the sound continues if the stimulation continues, until the patient's breath expires. The effect is an inability to speak inasmuch the stimulation causes a definite hesitation, though it does not completely stop the speech". This effect has been reported without any accompanying motor phenomena (Penfield and Boldrey, 1937).

All these studies highlight as vPM plays a role in motor control of speech, even if a detailed description is lacked.

Broca's area/BA44-45

Speech has been historically attributed to a complex network involving, as essential component, the *partes opercularis* and *triangularis* (Brodmann Area BA44 and 45 respectively) of the posterior inferior frontal *gyrus*. The observation of a lesion of areas BA44-45 in brains of patients affected by permanent "speech loss", reported by Pierre Paul Broca (1861), consecrated this region as the Broca's area, a crucial hub in the neural control of speech production (Berker et al., 1986; Amunts et al., 1999). As stated above, the "speech loss" in Broca's patients, described as the inability to *articulate language*, was termed *production* or *Broca's aphasia*. The ambiguity of the definition of "production" aphasia and its direct identification with the executive articulation of speech sounds, lead to

dogmatically consider, for almost a hundred years, Broca's area as the crucial area for motor control of speech, despite no evidence of motor output from this area has ever been provided. In the last decades, however, different studies raised a criticism on this claim, supported by two main observations: on one side the pure lesions of Broca's area do not result, as expected, in production aphasia, but rather in a transitory, rapidly improving mutism (Mohr et al., 1978) and, on the other side, the detailed analysis of the preserved brains of Broca's patients showed that the lesion was not actually confined to BA44-45, but involved other structures beyond the frontal operculum (Dronkers et al., 2007), challenging the univocal correlation between the production aphasia and the Broca's area and, importantly, the putative motor properties of Broca's area.

Only DES allowed a direct investigation of the functional properties of Broca's area. The deficit induced by stimulation of BA44-45/Broca's area during language tests impairs the speech performance, a phenomenon called "speech arrest*"* (Luders et al., 1987; Axelson et al., 2009; Chang et al., 2011, 2015, 2016**, Fig. 1.25**) and described as "the complete interruption of ongoing speech in absence of oro-facial movements and vocal output" (Petrovich et al., 2005; Tate et al., 2014, 2015; Chang et al., 2011, 2015, 2016; Tomasino et al., 2014; Havas et al., 2015). This effect is also described when DES is applied onto neighbouring vPM (see above and **Fig. 1.26**).

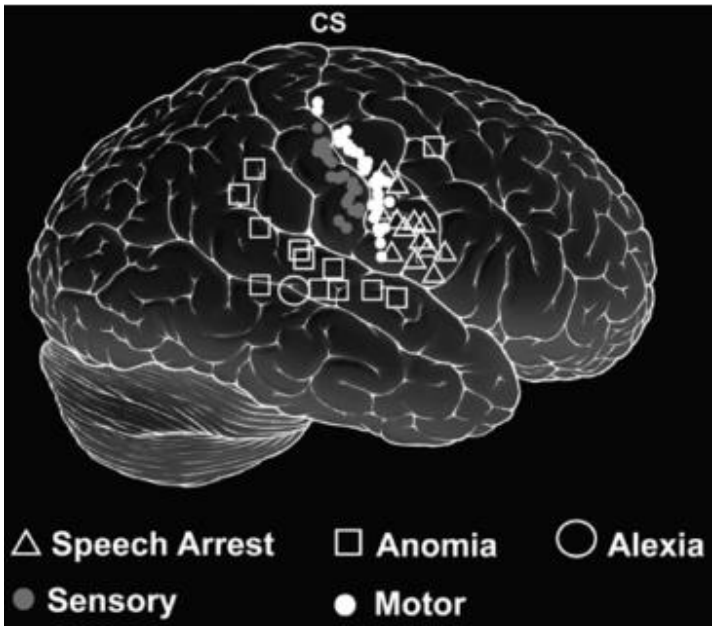

Figure 1.25 Composite of all positive motor, sensory, and language sites indentified by direct electrical stimulation on both hemispheres. CS: central sulcus.

From *Chang et al, 2011*.

Many studies attributed to Broca's area a higher cognitive role in language function, including semantic, phonological and syntactic processing. In the last decade both neuroimaging (with PET, fMRI, Moore and Price, 1999; Van Turennout et al., 2000) and electrophysiological studies (Ojemann et al., 1989; Schaffler et al., 1993; Levelt et al., 1999; Salmelin et al., 2000) investigated the language neural network in ecological condition (Indefrey and Levelt, 2004; Indefrey 2011; Hagoort and Indefrey, 2014) mainly by using picture naming tasks. In these studies, the activation of BA44-45 is observed in the time window between the picture presentation (about 455ms delay) and the motor articulation of the word (about 600ms delay, **Fig. 1.15**), suggesting its involvement mainly in intermediate phase of syllabification, i.e. a process needed for the phonological encoding of the word to be successively properly articulated in sounds (Indefrey and Levelt 2004). Within a putative functional subdivision of Broca's area, a phonological function is hypothesized for the posterior and most dorsal sector (BA44) and a semantic function for the anterior and most ventral sector (BA45) (Costafreda et al., 2006; Katzev et al., 2013; Bourguignon 2014). The latter, however, is only partly supported by recent literature reporting that lesions affecting inferior frontal *gyrus* result in phonological deficits (Robinson et al., 2012) without a clear impairment in semantic fluency (Henry and Crawford, 2004). Evidences suggest that Broca's area activation is not concurrent to the timing corresponding to conceptual preparation and to lemma selection (Indefrey and Levelt in 2004; Hulten et al., 2009; Flinker et al., 2015). As a possible criticism, these data were acquired in picture naming

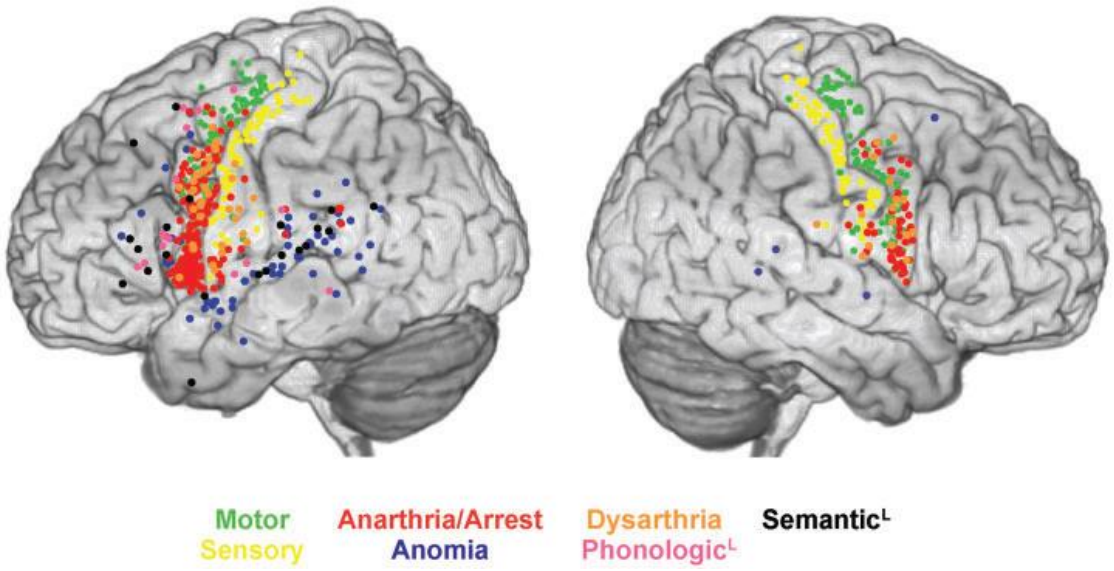

Figure 1.26 Compilation of all stimulation data in the right and left hemisphere demonstrating the wide distribution of cortical representation within and between critical functions of the human brain: motor (green), sensory (yellow), anarthria/speech arrest (red), anomia (blue), dysarthria (orange), phonologic (pink), semantic (black). L = left hemisphere only.

From *Tate et al., 2014 modified.*

tasks, and thus requiring the production of single words. Parallel studies requiring the performance of entire sentences (Demonet et al., 2005) reported that the activity of BA45 emerges highly significant suggesting its involvement in higher order semantic processes (Hagoort, 2005; Vigneau et al., 2006; Price, 2010; Friederici, 2011). The BA44 is, on the other hand, considered involved both in the phonological processing (Duffau et al., 2002; Costrafreda et al., 2006; Katzev et al., 2013) and syntax processing (Price 2010; Vigneau et al., 2006; Friederici 2011). Moreover, the ventral BA44 seems involved in "phonetic encoding" of words (Hickok and Poeppel 2004; 2007; Papoutsi et al., 2009), supported by the ''map of speech sounds'' (i.e. a topographic representation of phonemes or frequent syllables) described within the ventral BA44 (Guenther et al., 2006) and by the evidence of its activation clearly time-locked to the phonemic and articulatory representations preceding the speech articulation (Long et al., 2016), in single word tasks not requiring semantic and syntactic processes (e.g. repeating, reading; Flinker et al., 2015**, Fig. 1.27**).

Conclusively, although the involvement of Broca's area in language network is widely accepted, the exact role of this area in this complex function remains obscure. The most debated issue is its actual

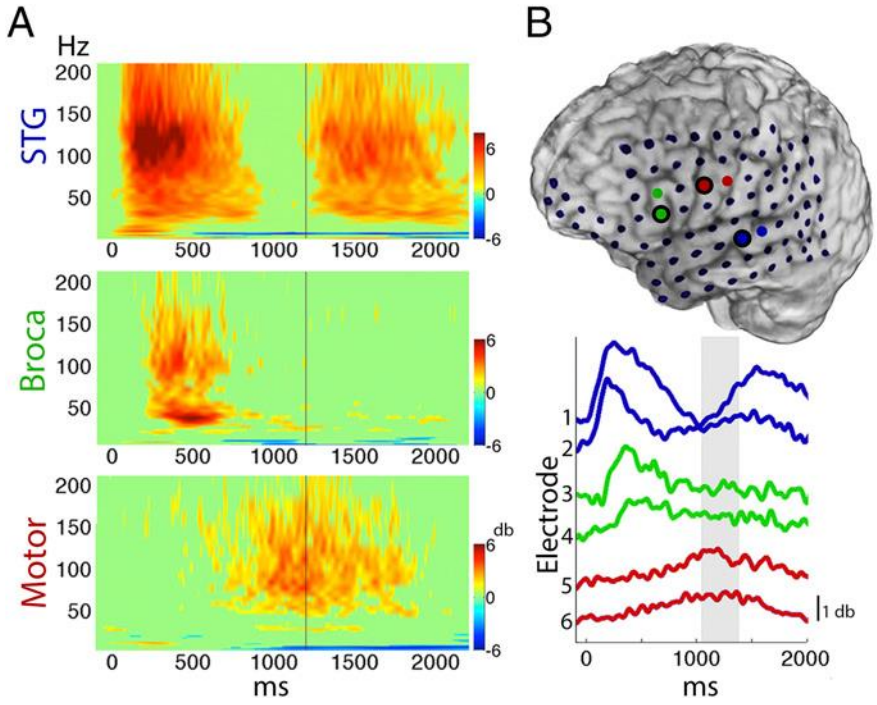

Figure 1.27 Repetition of monosyllabic words in a subject. **A)** Event-related spectral perturbations, averaged across trials, and locked to the onset of auditory word stimulus. Cortical activation indexed by power increases in high frequencies is first apparent in superior temporal gyrus (STG) during the word perception, subsequently in Broca's area, and finally extends to motor cortex during word production (vertical lines mark mean articulation onset). **B)** High frequency power (γhigh, 70–150 Hz) traces, averaged across trials, and locked to word stimulus onset are shown for STG (blue), Broca (green), and motor (red) electrodes. The first electrode in every pair is marked by a black circle and corresponds to the event-related spectral perturbations plotted on the left. The shaded grey area marks the distribution of articulation onset for this subject.

From *Flinker et al., 2015*.

involvement in motor control of speech, significantly challenged by the most recent neurophysiological studies showing a lack of evidence of Broca's activation concurrent to word's articulation (Flinker et al., 2015).

Focus of the PhD project was to investigate three out of five cortical areas involved in motor control of speech production, or rather Broca's area (BA44-45), vPM (vBA6) and M1 (BA4). However, also the supplementary motor area (SMA) and the insula cortex (BA13) program the speech. A brief description related to role of these two cortices in speech function is reported.

S*upplementary motor area*

Studies by K. Brodmann revealed that SMA, such as M1, has a motor cortical somatotopy in anterior-posterior direction: face, upper and lower limb (Chainay et al., 2004). SMA plays an important role in the planning, initiation and learning of complex motor functions (Penfield and Welch, 1951; Orgogozo and Larsen, 1979; Luders, 1996; Krainik et al., 2001; Nachev et al., 2008; Hiroshima et al., 2013), controlling contra- and ipsilateral muscles, and in particular in the dominant hemisphere is involved in the "initiation of speech" (Botez and Barbeau 1971; Crosson et al., 2001; Krainik et al., 2003). fMRI studies provided that SMA is activated during different language tasks, such as repetition and silent verbal fluency (Crosson et al., 2001; Krainik et al., 2003). Lesions within to the anterior portion of SMA induced a reduced spontaneous self-initiated speech, in the absence of any central-motor disorders of the vocal tract muscles and any deterioration of language functions (Ackermann and Riecker, 2010). Stimulation studies of the SMA anterior portion revealed that the stimulation produced facial movements (Luppino et al., 1991; Ikeda et al., 1992; Allison et al., 1996) and a not-motor phenomenon such as "speech arrest" (Fried et al., 1991). The stimulation sites that evoked facial movements were less than 10% on total points. Interestingly, the representation of articulatory speech movements seemed to be much greater than that of simple oro-facial movements, since many of these sites produced "speech arrest".

Insula cortex/BA13

The insula cortex is difficult to investigate. Until now, it is known that in its ventral-rostral portion, in human and in monkey, facial movements are represented (Showers and Lauer, 1961; Jezzini et al., 2012). In this region, in human, a recent meta-analysis (Oh et al., 2014) demonstrated that the left insula shows a preferential activation in response to speech production tasks. However, how this area is involved in motor programming, it is not clear.

Corticobulbar system

The corticobulbar tract (CBT) is a system connecting, through the pyramidal system, the cortical neurons controlling oro-facial muscles to their motor nuclei in the brainstem. (**Fig. 1.28**, Dick et al., 2014). The cortical region originating the largest number of corticobulbar fibres is the ventral portion of M1/BA4, an essential area for voluntary control of oro-facial and larynx muscles. A contribute to cortical control of these muscles originates also from the ventral pre-motor cortex (vPM/vBA6) and from the supplementary motor area (SMA). These areas most likely act indirectly by shaping the M1

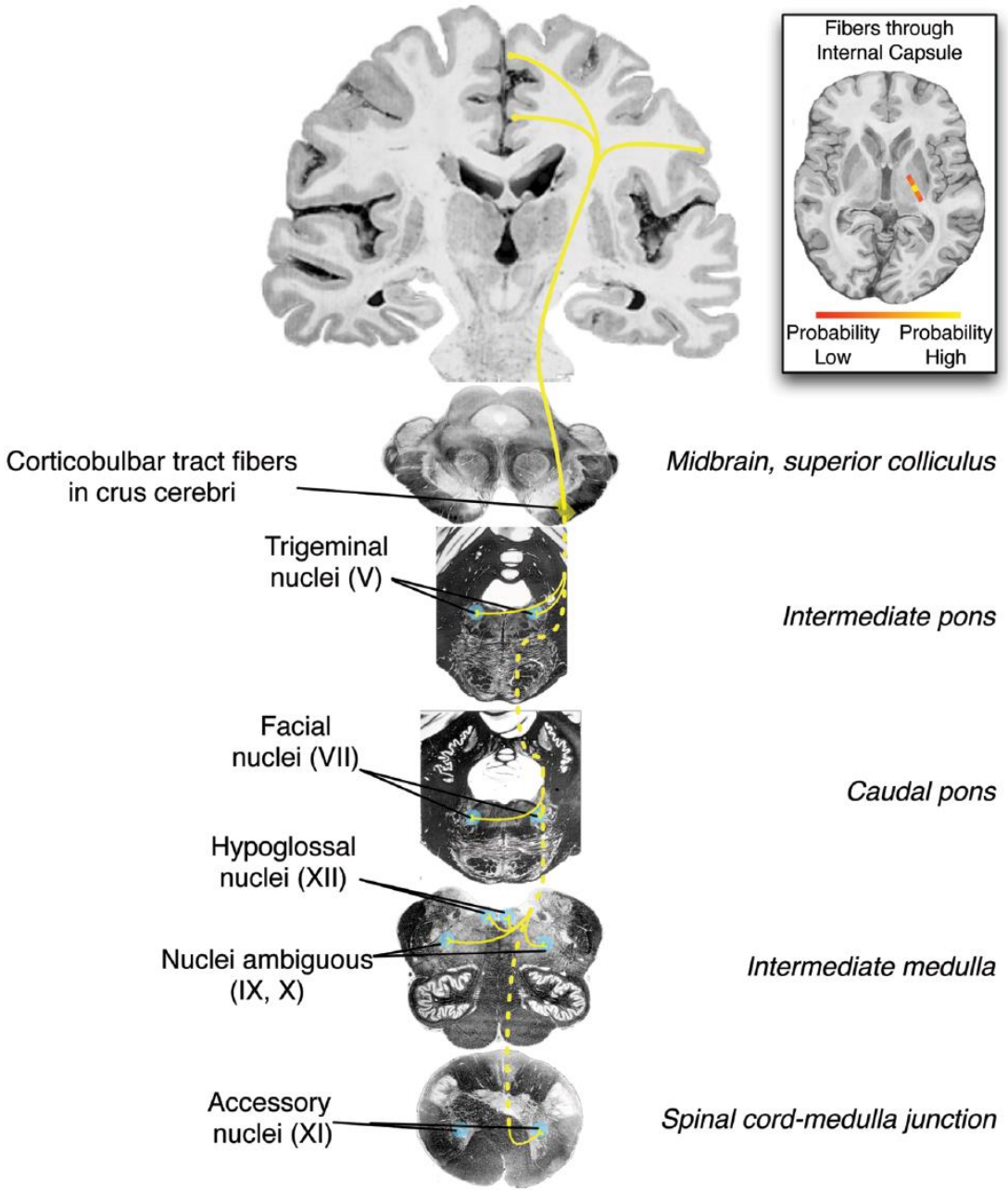

Figure 1.28 Descending fibres and nuclei of CBT relevant to speech. Inset shows the approximate pathway of fibres through the posterior limb of the internal capsule in axial view. Dotted projections indicate the approximate pathway of the tract through the brainstem.

From *Dick et al., 2014 modified.*

activity on phono-articulatory muscles, by means of cortcio-cortical projections (Dum and Strick, 1991). From these areas, the cortical neurons run in the corona radiata, in the *genu* of the ipsilateral internal capsule, and, at midbrain level, in the ventral cerebral peduncle (*crus cerebri,* **Fig. 1.28**). From here, the fibres descend in the brainstem, to the pontine and medullary level, leaving the pyramidal tract dorsally. Then, most of the fibres crosses to the contralateral side. Ipsilateral and contralateral fibres run through the ventromedial pontine and medullary reticular formation to finally reach the motor nuclei of the cranial nerves (Jurgens, 2002; Dick et al., 2014): trigeminal (V), facial (VII), hypoglossal (XII), ambiguus (IX, X) (**Fig. 1.28**). The actions on motorneurons is probably mediated by interneurons actually identified in the trigeminal nuclei, in the parvocellular reticular formation and in the dorsal reticular nucleus.

CBT is not an exclusive system connecting M1 to the brainstem motor nuclei. Neuroanatomical studies showed massive projection from M1 to the ventral putamen. The ventral putamen projects into the dorsal-lateral *substantia nigra pars reticulate*, which, in turn, projects to the parvocellular reticular formation, directly connected with the phonatory motoneurons. The parvocellular reticular formation has been shown to be indeed a crucial structure for vocal production (Zhang and Sasamoto, 1990). Other two addition "extrapyramidal" pathways connect M1 with the parvocellular reticular formation: one via the deep layers of the lateral superior colliculus and the other via the dorsal red nucleus (Jurgens, 2002).

Not-human primate studies. At present only very few anatomical studies in primates investigated the origin of fibres that control the movements of the oro-facial muscles. Kuypers employed a degeneration tracing technique to examine descending projections to the lower brainstem following partial ablation of the cortex on cats, *Macaca* monkeys and *Pan apes* (Kuypers, 1958a,c), showing scarce direct cortico-nuclear projections to the facial nucleus in cats, relevant in *Macaca* and predominant in *Pan*. In the latter, the lateral and dorsal facial sub-nuclei receive a bilateral direct input from the pre-central cortex. A few decades later, Jenny and Saper employed horseradish peroxidase in *Macaca* to determine the differential distribution to the facial sub-nuclei of cortico-nuclear projections from the pre-central cortex of both sides (Jenny and Saper, 1987). Their findings indicated that cortico-nuclear projections were mostly directed to the lateral and intermediate sub-nuclei (containing motoneurons to perioral muscles) on both sides with an ipsilateral:contralateral ratio of about 1:3. Morecraft and colleagues (2001, 2004) showed that (**Fig. 1.29**) M1/BA4 and vPM/vBA6 were the origin of the majority of cortico-nuclear fibres to the facial nucleus. M1/BA4 was mainly connected to the contralateral lateral sub-nucleus hosting mainly the representation of the contralateral perioral muscles, while vPM/vBA6 projected, bilaterally, mainly to the lateral sub-nucleus. In particular, vPM projects to spinal trigeminal nucleus, the solitary tract nucleus and the

facial nucleus, while no vPM projections to the nucleus ambiguous were identified, suggesting a lack of direct connections with laryngeal motoneurons in non-human primates (Simonyan and Jürgens, 2003).

Mesial BA6 (SMA) projected axons mainly to the medial sub-nucleus bilaterally, i.e. to motoneurons innervating the *auricularis*, *frontalis* and *platysma* muscles. The caudal cingulate area contained a small contingent of neurons projecting exclusively to the sector of the contralateral lateral sub-nucleus where motoneurons innervating upper lip muscles are found.

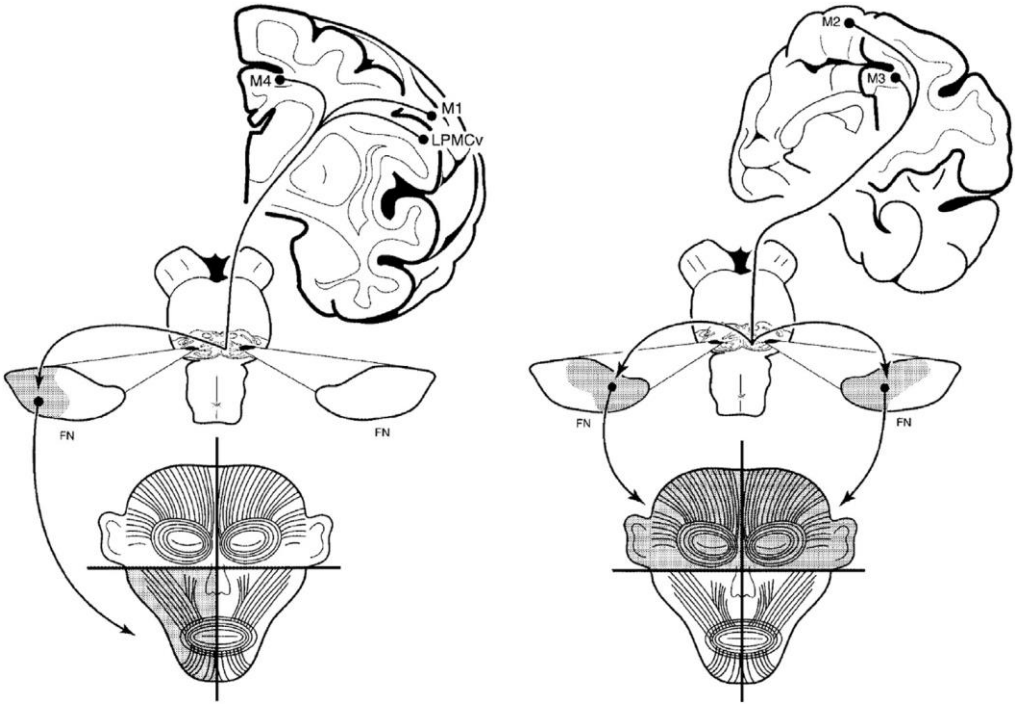

Figure 1.29 Summary of the results of the retrograde labelling study in the macaque monkey. Left panel: Cortical areas projecting to the lateral sub nucleus, which in turn innervates perioral muscles. Right panel: Cortical areas innervating motoneurons in the medial, intermediate and dorsal sub nuclei, which in turn innervate auricular, periocular and forehead muscles.
M1 = primary motor cortex; LPMCv = ventral premotor cortex. M4 = rostral cingulate motor cortex. M2 = supplementary motor area; M3 = posterior cingulate motor cortex.

From *Morecraft et al., 2001*.

Human primate. Comparative anatomy studies (Sherwood et al., 2004a, 2005; Diogo et al., 2009) suggest that the facial nucleus of human is larger than that of monkeys. Similarly to monkeys, it is suggested that the cortical control of the phono-articulatory apparatus originate mainly from M1, via CBT, while the action of vPM might be exerted over the activity of oro-facial and laryngeal muscles via the dense cortico-cortical connections with M1 *(*Tokuno et al., 1997; Simonyan and Jürgens, 2005). These cortico-cortical connections were explored in TMS (transcranial magnetic stimulation)

studies using the conditioning paradigm of short-latency intra-cortical inhibition and facilitation (Kujirai et al., 1993; Kobayashi et al., 2001; Paradiso et al., 2005; Pilurzi et al., 2013). These effects were actually demonstrated in *lip depressor* muscles, in the *mentalis* muscle (Kobayashi et al., 2001; Paradiso et al., 2005) and in a perioral muscle (the *depressor anguli oris*, Pilurzi et al., 2013) in both the ipsilateral and the contralateral motor representations in the motor cortex.
These studies suggest that if M1/BA4 has a direct connection with brainstem, vPM/vBA6 is connected with M1.

Peripheral apparatus for speech production

The peripheral apparatus is composed of cranial nerves and of phono-articulatory muscles. The cranial nerves emerge from their motor nuclei located in brainstem (trigeminal motor (V), facial (VII), hypoglossal (XII), ambiguus (IX, X) nuclei) to phono-articulatory muscles. The phono-articulatory apparatus is composed by about 100 muscles.

Cranial nerves

The trigeminal nerve or V cranial nerve

The trigeminal nerve is divided in three branching: ophthalmic, maxillary and mandibular nerves (upper image in **Fig. 1.30**). Each of them converge on the trigeminal ganglion (also called the semilunar ganglion or gasserian ganglion) located within Meckel's cave and containing the cell bodies of incoming sensory-nerve fibres. The ophthalmic and maxillary nerves are purely sensory, while the mandibular nerve has sensory and motor functions. From the trigeminal ganglion a single sensory root enters the brainstem at the level of the pons. Adjacent to the sensory root, a motor root emerges from the pons at the same level. Motor fibres pass through the trigeminal ganglion on their way to peripheral muscles, but their cell bodies are located in the nucleus of the trigeminal nerve, deep within the pons. These motor fibres (mandibular nerve) exit from the cranial cavity and enter in the upper part of the infratemporal hollow. Gradually, the nerve, crossing the oval hole, emits a collateral branch (the spinosus nerve) and then immediately it is split into two branches: the posteromedial and the anterolateral branch. The first branch innerves the *masseter*, the *temporal*, the *medial* and *lateral pterygoids* and the *buccinator* muscles. The second branch innerves the *tensor veli palatini*, the *mylohyoid*, the *anterior belly of the digastric*, the *inner pterygoids* and the *tensor tympani* muscles.

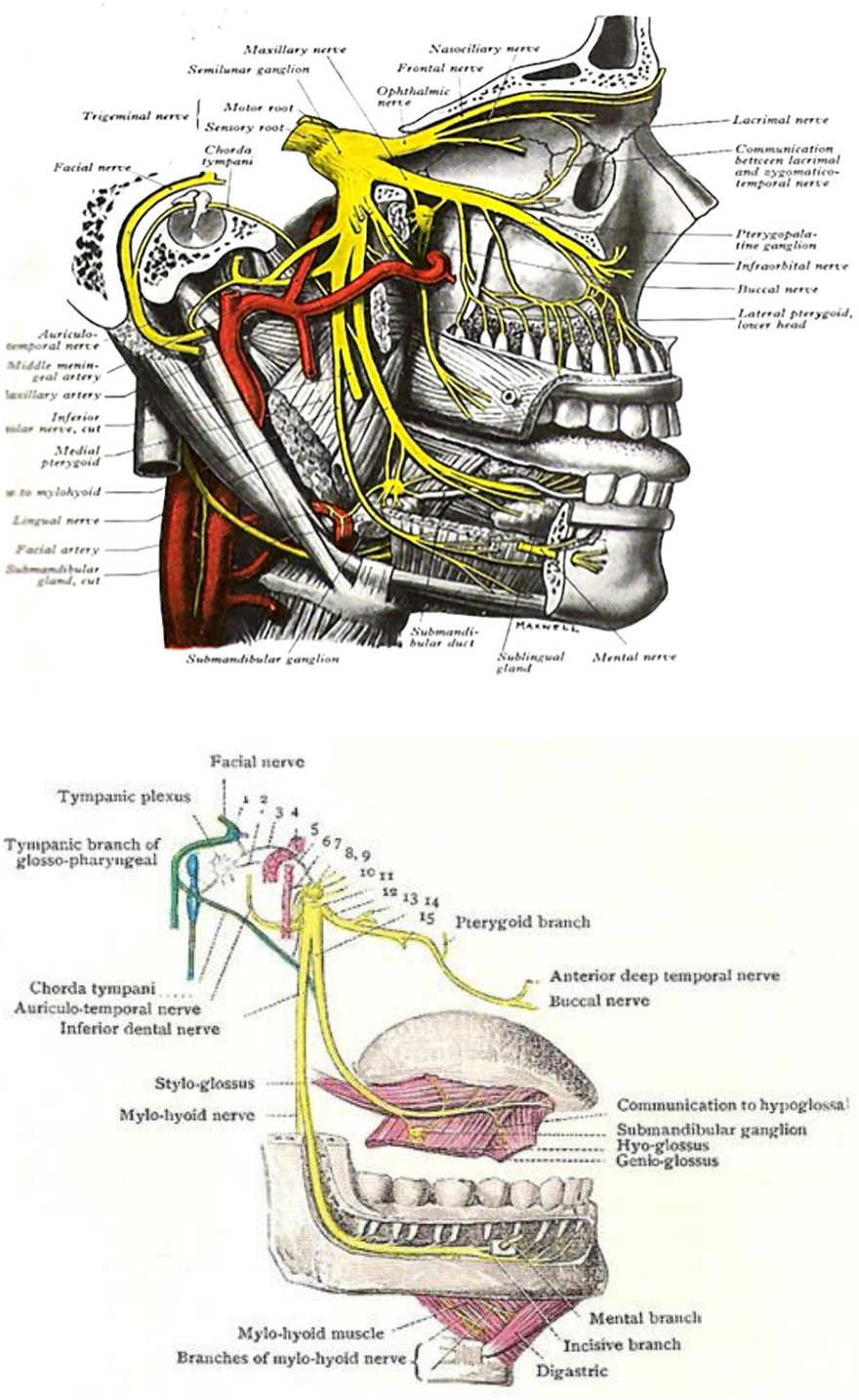

Figure 1.30 Schematic representation of the trigeminal nerve or V cranial nerve, of its branches and of its innervated-muscles.

The facial nerve or VII cranial nerve

The VII cranial nerve emerges from the brainstem in the ventral-lateral aspect of the ponto-medullary junction. Its path is commonly divided in three portions, the intra-cranial one, the intra-petrosal or

intra-temporal (running in the facial canal in the temporal bone) and the extra-cranial one. The motor branches of the facial nerve directed to facial muscles include one collateral branch, the posterior auricular nerve, which leaves the main trunk of the facial nerve as soon as it exits the foramen *stylomastoideus* and five terminal branches, the temporal, the zygomatic, the buccal, the marginal (or mandibular) and the cervical branches (**Fig. 1.31**). The territory of innervation of each facial nerve is confined to the ipsilateral half of the face, even in the case of the *orbicularis oris*.

The motor nucleus of the facial nerve is the largest of all motor nuclei of the brainstem. It is located in the caudal portion of the ventral-lateral pontine tegmentum and its efferent fibres contain alpha motoneurons directed to mimetic muscles, among which *orbicularis oris*, *mentalis*, *platysma*. The facial nucleus, in addition to alpha motoneurons, hosts interneurons, including the Renshaw cells, although no recurrent inhibition was describer in animal models.

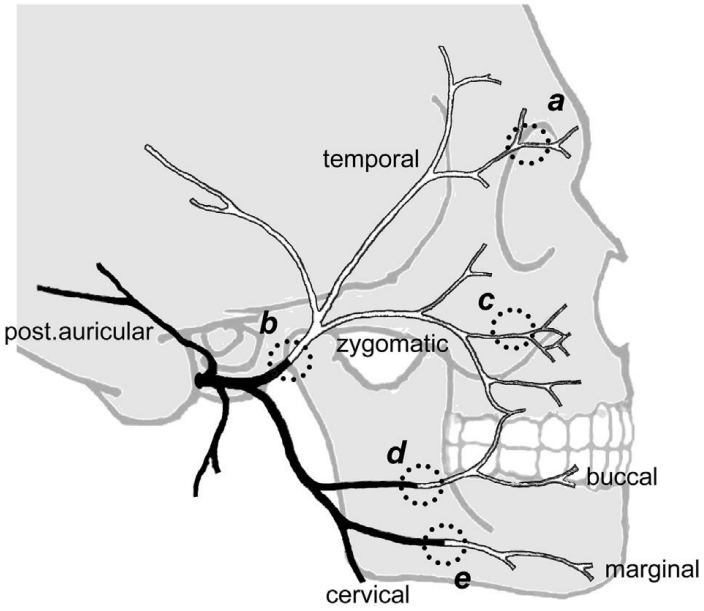

Figure 1.31 Representation of the extracranial course of the facial nerve, or VII cranial nerve, with labelling of its branches. Dashed circles represent the points of inlet of trigeminal anastomotic fibres to form trigeminal-facial anastomoses. The course of the facial nerve containing also trigeminal anastomotic fibres is highlighted in white.
Labels: a = supraorbital-temporal anastomosis;
b = auriculotemporal-facial anastomosis;
c = infraorbital-zygomatic anastomosis;
d = buccal–buccal anastomosis;
e = mentalmarginal anastomosis.

From *Cattaneo and Pavesi, 2014*.

The glossopharyngeal nerve or IX cranial nerve

The glossopharyngeal nerve is a mixed nerve that carries afferent sensory and efferent motor information. From the rostral part of ambiguus nucleus in the medulla oblongata, the nerve leaves the skull through the central part of the jugular foramen (**Fig. 1.32**). On the inferior side, the nerve is lateral and anterior to the vagus (X) nerve and accessory (XI) nerve. In its passage through the foramen (with X and XI cranial nerves), the glossopharyngeal nerve passes between the jugular vein and internal carotid artery, descending anterior to the latter. It then curves forward, forming an arch on the side of the neck and lying upon the *stylopharyngeus* and *middle pharyngeal constrictor* muscle.

From there, it is distributed to the palatine tonsil, the mucous membrane of the fauces and base of the tongue, and the serous glands of the mouth.

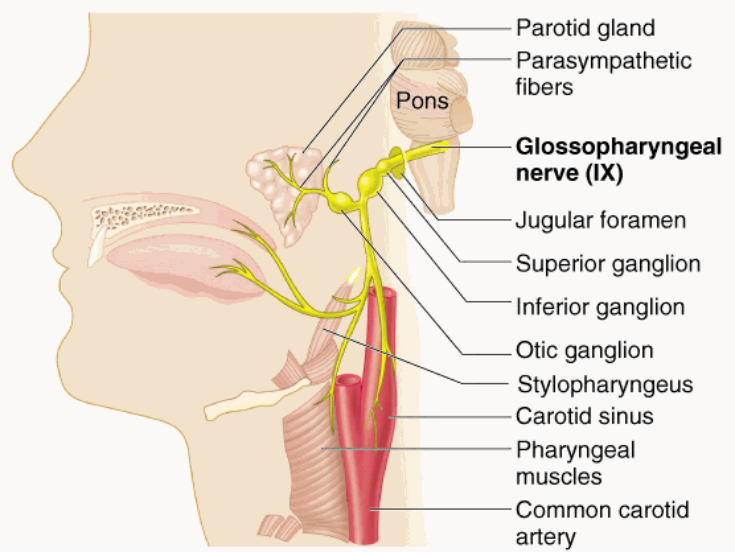

Figure 1.32 Schematic representation of the glossopharyngeal nerve or IX cranial nerve, of its branches and of its innervated-muscles.

The vagus nerve or X cranial nerve

The vagus nerve leaves the medulla oblongata and extends through the jugular foramen, then passes into the carotid sheath between the internal carotid artery and the internal jugular vein down to the neck (**Fig. 1.33**), chest and abdomen, where it contributes to the innervation of the viscera (not shown). The right vagus nerve gives rise to the right recurrent laryngeal nerve, which hooks around the right subclavian artery and ascends into the neck between the trachea and oesophagus, innervating

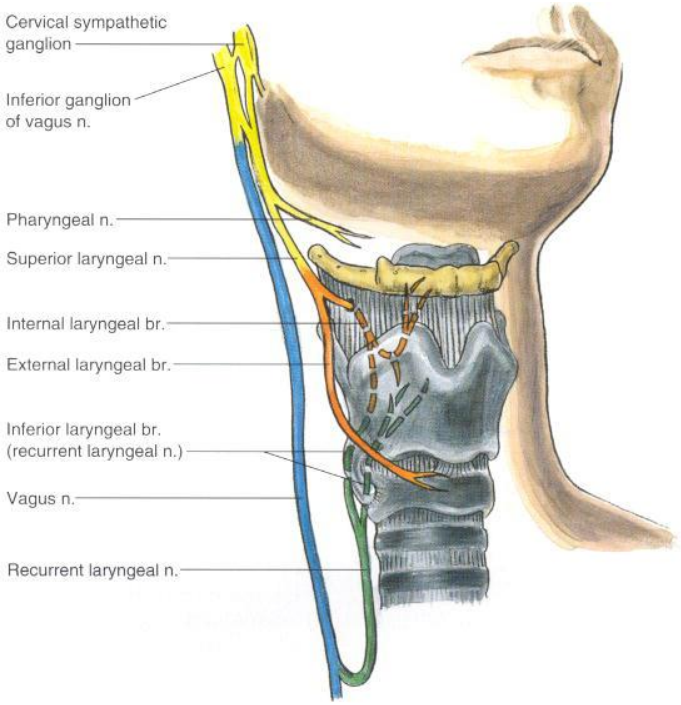

Figure 1.33 Schematic representation of the vagus nerve or X cranial nerve, of its branches and of its innervated-muscles.

the laryngeal muscles. The left vagus nerve enters the thorax between left common carotid artery and left subclavian artery and descends on the aortic arch. It gives rise to the left recurrent laryngeal nerve, which hooks around the aortic arch to the left of the ligamentum arteriosum and ascends between the trachea and esophagus, reaching the laryngeal muscles. The left vagus enters the abdomen as the anterior vagal trunk in the esophageal hiatus of the diaphragm.

The hypoglossal nerve or XII cranial nerve

The hypoglossal nerve is a pure motor nerve that innervates all extrinsic and intrinsic muscles of the tongue. The nerve arises from the hypoglossal nucleus in the brainstem, travels close to the vagus nerve (X) and spinal division of the accessory nerve (XI) and passes between the internal carotid artery and internal jugular vein. After passing to the submandibular region, passes upwards and anteriorly on the *hyoglossus* muscle. It passes up on the outer side of the *genioglossus* muscle and continues in a forward direction to the tip of the tongue. It distributes branches to the intrinsic and extrinsic muscle of the tongue (**Fig. 1.34**).

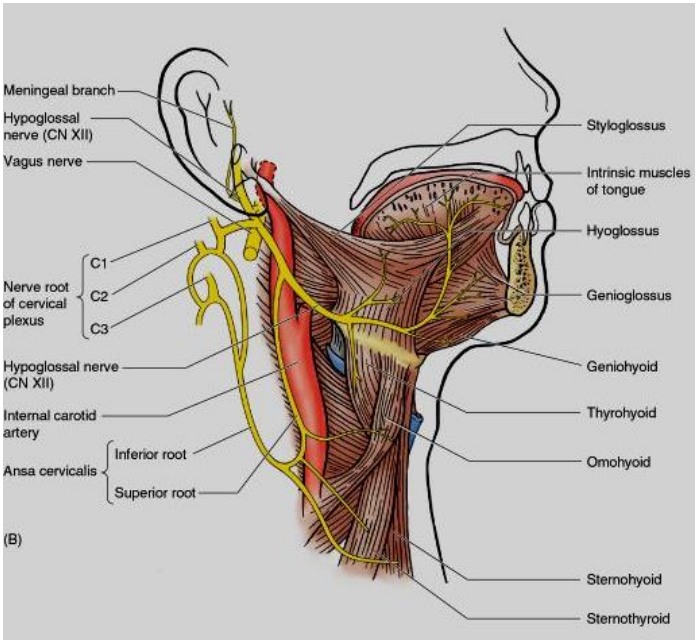

Figure 1.34 Schematic representation of the hypoglossal nerve or XII cranial nerve, of its branches and of its innervated-muscles.

Phono-articulatory system

Speech is a motor activity performed by the phono-articulatory apparatus, which is composed by about 100 articulatory muscles (Kent, 2000). These muscles can be clustered into five interacting functional sub-systems sub serving:

- respiration (thoracic and abdominal muscles)
- phonation (intrinsic and extrinsic laryngeal muscles)

- sound resonance (pharynx and supra-laryngeal structures)
- articulation (supra-laryngeal structures)
- addition of supra-segmental features

Respiration. The airflow from the lungs (in expiration) is necessary to generate voice and, consequently, speech. The main inspiratory muscle is the diaphragm, which increases the volume of the thorax and compresses the abdominal cavity as it contracts. By creating a negative pressure (i.e., sub-atmospheric) in the chest, causing air to flow in and fill the lungs. During a normal passive expiration, the diaphragm relaxes and its recoil reduces the volume of the thorax, leading to expiration. In more forceful inspiration and expiration, a number of other costal and abdominal muscles may be recruited. Among the abdominal muscles, the internal and external oblique muscles are activated before the *transversus* and *rectus abdomini* muscles. The activation of the obliqui muscles and the *transversus* muscles is well correlated in their activity with phonation, (**Fig. 1.35**, Jurgens, 2002). The motoneurons innervating the abdominal muscles lie in the ventral horn of the thoracic and upper lumbar spinal cord, while the motoneurons innervating the diaphragm are located in the phrenic nucleus, localized in the C3–C5 spinal segments (Routal and Pal, 1999).

Phonation or sound production. When the vocal folds in the larynx are in position for the phonation (i.e. adducted), the air-stream from the lungs makes the soft cover of the vocal tract to vibrate. This generate a sound that is raw (although already not pure) and un-modulated. In order to become speech, this raw signal must be filtered in the pharynx and in the oral cavity. Control of vocal fold movements is exerted by five intrinsic laryngeal muscles (**Fig. 1.35**). The motoneurons innervating the intrinsic laryngeal muscles are located in the nucleus ambiguus in the lateral tegmental field of the medulla, while the motoneurons innervating the extrinsic laryngeal muscles are located in C1-C2 spinal segments.

Resonance. As the sound passes from the larynx into the pharynx, the sound produced by vocal folds is amplified and filtered from:

- the pharynx that, providing a resonating cavity, helps shape the sound for the speech. The motoneurons innervating the muscles of the pharynx are located in the nucleus ambiguus in the lateral tegmental field of the medulla;
- the velopharyngeal sphincter mechanism that regulates the nasality of the speech sound. To produce nasal sounds such as /n/ or /m/, the velopharyngeal sphincter is opened by lowering the velum or soft palate so that the sound goes through the nose. To produce oral sounds, the velum must be elevated to close the velopharyngeal sphincter so that no air goes through the nose. The position of the velum is determined by the *levator* and *tensor veli palatini* muscles. The *levator veli palatini* is

innervated from the ambiguus nucleus via glossopharyngeal nerve, while the *tensor veli palatini* muscle receives its innervation from the trigeminal motor nucleus via mandibular nerve.

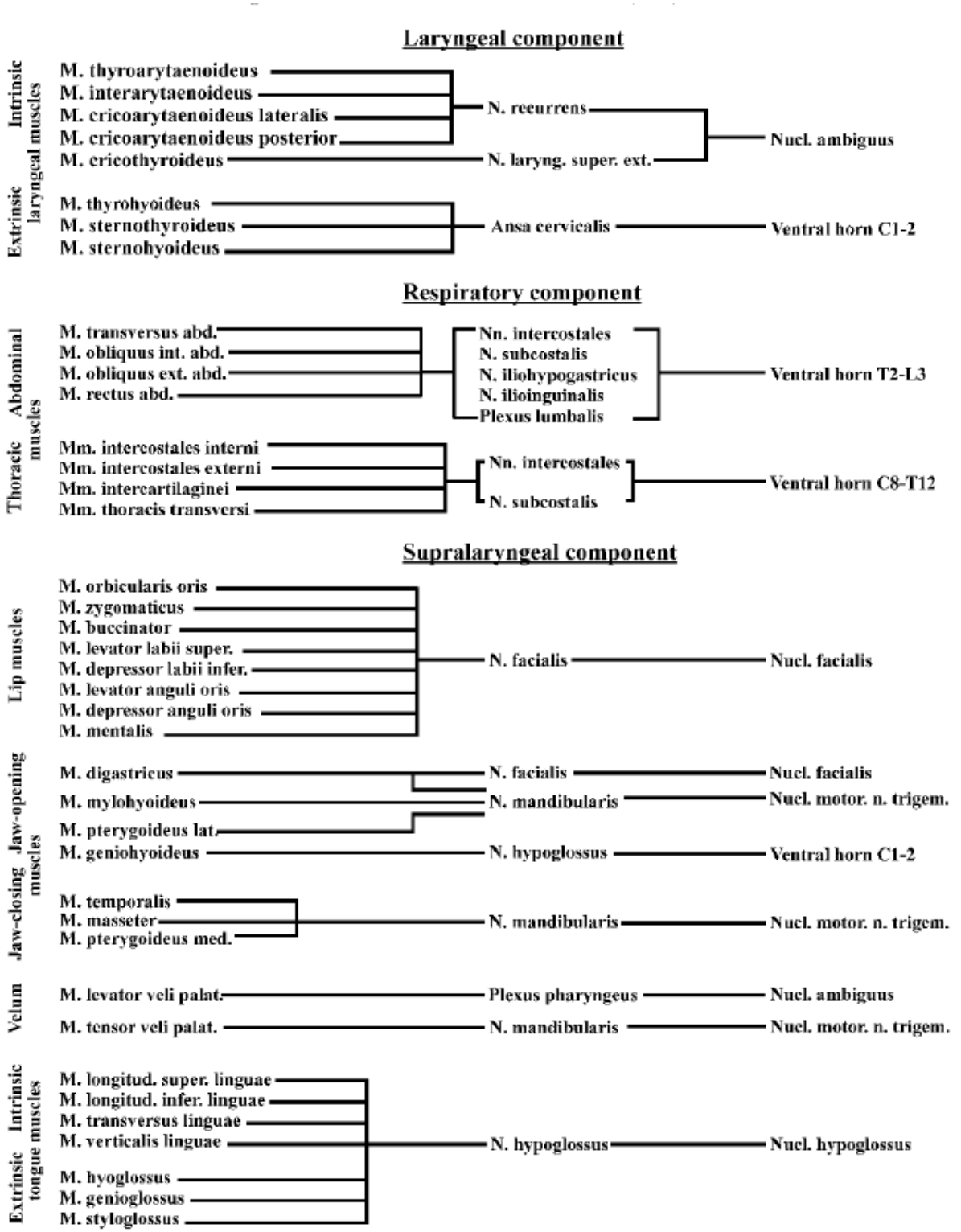

Figure 1.35 Scheme of phonatory muscle innervation. On the left the muscles are listed; in the middle the peripheral nerves innervating the muscles are indicated; on the right the sites of the corresponding motoneurons are given.

From *Jurgens, 2002.*

Articulation. In order to produce the large variety of different speech sounds, the sound stream that has been emitted from the vocal folds and filtered in the pharynx, is further modified through multiple quick movements or also called gestures by the phono-articulators in the oral cavity. For example, the most consonant sounds are oral speech sounds, i.e., produced in the oral cavity. However, many languages of the world also use laryngeal and pharyngeal consonants. For instance, some British dialects of the English language substitute a little laryngeal clicking noise for word-medial and final "t"-sounds, so that "butter" is pronounced "bu'er".

Supra-laryngeal muscles (**Fig. 1.35**) modulate the length and shape of the supra-laryngeal tract. The length of the vocal tract can be increased by protruding the lips (activating of the *orbicularis oris* muscles together with the *mentalis* muscle) or lowering the larynx. The vocal tract can be shortened by drawing back the mouth corners or lifting the larynx (activating the *mylohyoid* and *thyrohyoid* muscles). The muscles controlling length of the vocal tract are innervated by the facial and mandibular nerves and the *ansa cervicalis*.

Another factor significantly affecting the sound/voice resonance is the degree of mouth-opening. Jaw-opener muscles are the *digastricus, mylohyoid, pterygoid lateralis* and *geniohyoid* muscles, while jaw-closer muscles are *temporalis, masseter* and *pterygoid medialis* muscles. *Pterygoid* and *digastricus* muscles are innervated by the trigeminal motor nucleus via trigeminal (mandibular) nerve. *Temporalis, masseter* and *mylohyoid* muscles are innervated by trigeminal nerve.

Another important muscle in speech, is the tongue, controlled by intrinsic and extrinsic muscles. These muscles are innervated by hypoglossal nerve (Holstege et al., 1983; Dobbins and Feldman, 1995).

Supra-segmental characteristics. This subsystem comprises features of speech such as pitch, inflection and rhythm of speech and it is also referred to as prosody or speech melody. Beyond the linguistic content of a phrase, in fact, the emphasis that a speaker puts on words gives important information about his or her emotional and physical state.

Articulation and oral cavity

When speech sounds are formed in the oral cavity, the air and the sound stream from the larynx is modified by articulating muscles. These phono-articulatory structures can be classified as *active* or as structures that provide a *passive* counterpart to the movement of an active articulator. The first are represented by the lips, the mandible, the tongue and the soft palate. The second are the front teeth, the alveolar ridge and the hard and soft palate (**Fig. 1.36**). The soft palate serves as both an active and a passive articulator. It has an active function because it regulates the nasal-oral balance during speech

and serve as a passive counterpart for the tongue when we say a sound like “k”. In all spoken language, these articulatory muscles modulate the airflow to produce the consonant and vowel sounds. The consonant sound is usual characterized by the position of phono-articulatorys and whether it is voiced or not. For instance, the sound /f/ is produced by bringing the lower lip close to the upper incisors. If we then switch on our vocal folds, we get the sound /v/. So, /v/ is a voiced sound while /f/ is an unvoiced sound. The vowels are continuous, harmonic sounds that are produced with different degrees of tongue elevation, jaw opening and lip rounding. Every syllable in speech is constructed around a vowel. The vowels amplify determined resonance frequencies of the raw glottal sound. /a/ like in “car”, /i/ like in “beet” and /u/ like in “boot” are the most extreme positions of vowel articulation that we can assume. In /a/, the tongue is flat again the floor of the mouth and the jaw and lips are opened maximally. In /i/, the tongue is elevated towards the hard palate. The jaw is elevated relatively high and the lips are slightly spread. In /u/, the tongue is raised towards the velum. The jaw is elevated again but this time, the lips can produce such as /e/, /o/ etc. are somewhere in between these extreme positions.

There are many degrees of freedom for the active articulators during speech production: whether the tongue approximates the alveolar ridge or goes to the post-alveolar region, which is just a few millimetres posterior, causes two markedly different sounds: /s/ vs /ʃ/, the “sh” sound.

The adequate articulatory gestures require the highly skilled control of the coordination of all the sub-systems of the speech motor apparatus: the respiration, the phonation, the resonance and the articulation, as well as the supra-segmental characteristics. An adult can produce speech rates of up to 250 words per minute, which corresponds to six to nine syllables per second. We are able to

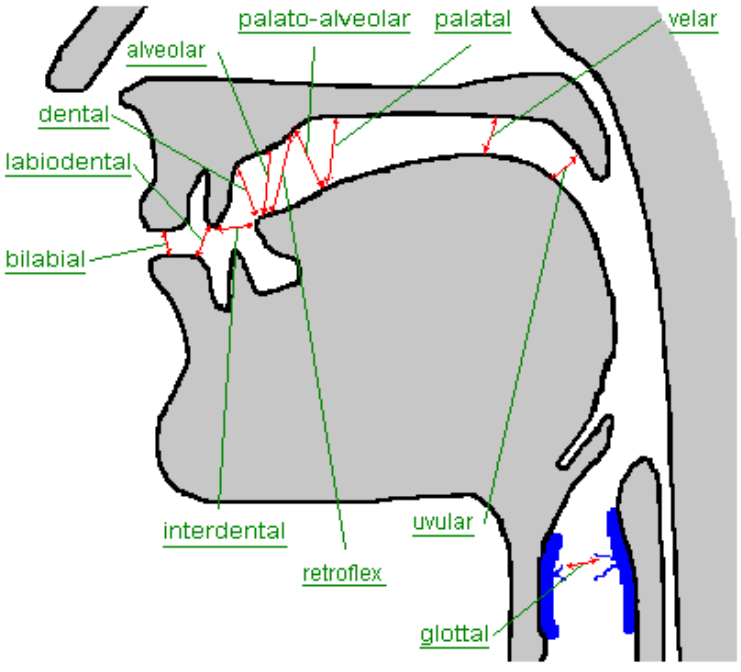

Figure 1. 36 Schematic representation of the places of articulation.

produce so many sounds at this high rate because we blend them together in *co-articulation*. (see above Liberman's study). The acquisition of the mature neural control of phono-articulatory apparatus allowing correct speech production takes years in childhood.

The oro-facial muscles involved in speech production

*Innervated muscles by facial nerve***.** The muscles innervated by the VII (facial) cranial nerve are a group of skeletal muscles that originate from the second pharyngeal arch. They form a complex system and contribute significantly to human behaviour in a wide range of functions as speech production, but also feeding and the communication of affective states (Cattaneo and Pavesi, 2014). The facial muscles are conventionally classified as individual muscles, each with its own identity and function. The **Figure 1.37** shows a schematic representation of human facial muscles according to the most frequent nomenclature. The facial muscles involved in speech production are located in the perioral region that is limited cranially by the zygomatic arch and caudally by the lower margin of the mandible. Its anatomy is complex and very variable. It is recognized a deep and a superficial muscular layer, which are separated by a plane containing the branching of the facial nerve. The deep layer contains the deep part of the *orbicularis oris* muscle and the *buccinator* muscle. The *orbicularis oris* is composed of four independent quadrants that interlace and give only an appearance of circularity (**Fig. 1.38**). It consists of numerous strata of muscular fibers, surrounding the mouth, with different direction clustered in four quadrants. It consists partly of fibers derived from the other facial muscles, inserted into the lips, and partly of fibers proper to the lips. Of the former, a considerable number are derived from the *buccinator* muscle and form the deeper stratum of the *orbicularis oris*. The *buccinator-orbicularis oris* system, from both sides, constitutes the basis of the cheeks. It is solidly anchored posterior to the pterygomandibular raphé and the adjacent mandibular and maxillary bones and is robustly connected to the other side of the face by means of the deep *orbicularis oris* fibres, thus forming a sling that literally holds the mouth and cheeks in place. The system acts by pressing the cheek against the teeth, thus providing active containment of the oral cavity. Given its structural function, its morphology is fairly stable across individuals of the human species (D'Andrea and Barbaix, 2006). The superficial perioral muscles on the contrary, show an astounding degree of intersubject variability. The muscles in the superficial layer are organized in a hub and spoke pattern around the oral orifice. By contracting, they act on the lips and perioral skin along radial trajectories centred on the oral orifice. The allowed movements belong therefore to two antagonistic classes: centripetal movements determining lip closure and centrifugal movements determining lip opening. Centripetal movements are carried out mainly by the *orbicularis oris* muscle, with the aid of *mentalis* muscle (**Fig. 1.38**). The *mentalis* arises from the mandibular bone and terminates in the skin of the

chin, which it elevates when contracting, ultimately producing a pout-like posture. Centrifugal movements are produced by contraction of a series of muscles disposed radially around the mouth. In the upper perioral region, they are: the *risorius*, the *zygomaticus major* and *minor*, the *levator labii superioris* and the *levator labii superioris alaquae nasi* muscles. The *risorius* muscle is considered to be a prolongation of the *platysma* muscle reaching the corner of the mouth. In the lower perioral region they are: the *depressor anguli oris*, the *depressor labii inferioris* (Cattaneo and Pavesi, 2014) and *platysma*. Their contraction lowers the lower lip. The *platysma* concurs in part to centrifugal movements of the lateral lower lips but it extends well beyond the perioral region (**Fig. 1.38**), into the anterior part of the neck. It consists in longitudinally oriented fibres attached caudally to the subclavear skin and cranially to the mandible margin and to the lateral aspect of the lower lips, becoming the *risorius* muscle (Cattaneo and Pavesi, 2014).

The facial muscle fibres, as all the skeletal muscle fibres, are classified into type I, type IIA and type IIB. The fibre typing provides the signature of fundamental physiological properties of muscles, namely the velocity of contraction, the maximal tension, and the susceptibility to fatigue. The relative composition of fibre types of a single muscle is therefore an important indicator of its function in behaviour. As a generalization, a dominance of type I fibres (slow twitch – fatigue resistant fibres) indicates that a muscle is involved in prolonged "postural" movements. Conversely, the prevalence of type II (fast twitch) fibres is the appropriate equipment for fast, phasic contractions. Among the

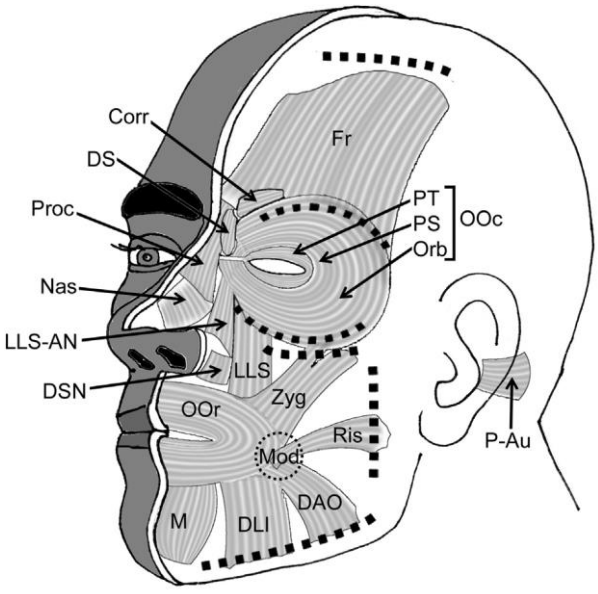

Figure 1.37 Schematic representation of the facial muscular system. Corr = *Corrugator*; Fr = *Frontalis*; Proc = *Procerus*; Nas = *Nasalis*; DS = *Depressor Supercilii*; OOc = *Orbicularis Oculi*; PT = *Pretarsal*; PS = *Preseptal*; Orb = *Orbital*; LLS-AN = *levator labii superioris alaquae nasi*; LLS = *levator labii superioris*; DSN = *depressor speti nasi*; Zyg = *zygomaticus major* and *minor*; Ris = *risorius;* M = *mentalis*; DLI = *depressor labi inferioris*; DAO = *depressor anguli oris*; P-Au = *posterior auricularis*. Bold dashed lines represent the main points of tethering of the facial skin to the skill. Mod = *modiolus*.

From *Cattaneo and Pavesi, 2014*.

main facial muscles above indicate, the *platysma*, the *mentalis*, the *depressor anguli oris*, the *orbicularis oris*, the *levator anguli oris*, the *zygomatici*, and the *levator labii superioris* are involved in speech production and they have an approximate fast-slow fibre type ratio of 2:1 (Freilinger et al., 1990). Moreover, the fibre types are not uniformly distributed within single muscles but are rather clustered in sub-fascicles. For example the inner most fascicles of the *orbicularis oris* muscle are entirely formed by type I fibres in contrast with the remaining muscle where type I fibres represent only the 30% of the total (Freilinger et al., 1990), indicating a functional subdivision within the muscle. The inner part is equipped for the tonic contraction of sphincteric activity. The outer part is involved in more dynamic movements.

Innervated muscles by trigeminal nerve. The facial muscles innervated by the V (trigeminal) cranial nerve, involving in speech production, are a group of skeletal muscles, also involved in biting, chewing, swallowing. These muscles are: the *masseter*, the *temporal* and the *medial* and *lateral pterygoids* –principally masticatory muscles-, and the *tensor veli palatini*, the *mylohyoid*, the *anterior belly of the digastric,* the *tensor tympani*. Among these muscles, the *anterior digastric* and *mylohyoid* (Holstege and Subramanian, 2015) are located in the lower perioral region and lower the lower lip following to their centrifugal movement during speech production (**Fig. 1.38**, Holstege et al., 1983). In particular, the *mylohyoid* muscle is a paired muscle running from the mandible to the hyoid bone, forming the floor of the oral cavity of the mouth. It is flat and triangular, it is situated superior to the *anterior belly* of the *digastric* muscle, it is a pharyngeal muscle and it controls the tongue movements. This last process controls the part of the tongue that makes velar consonants — such as the “g” in “good” and the “k” in “king” — and vowels.

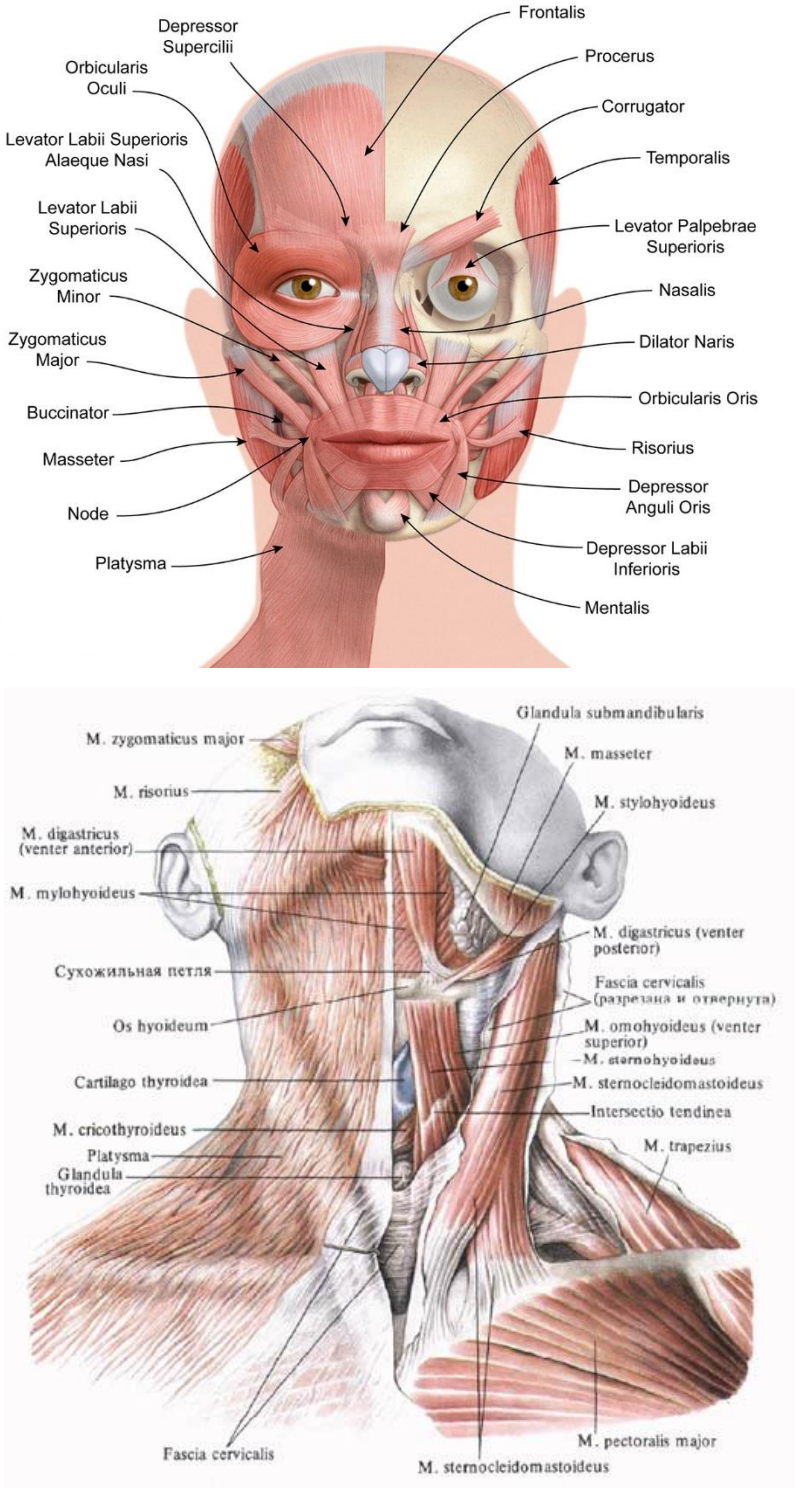

Figure 1.38 Facial muscles.

1.4 AIM OF PhD PROJECT

As described in details in Introduction, the language, as form of verbal communication –and of thought-, is a unique human ability. It is complex form of communication, consisting of different levels of representation (phonological, syntactic and semantic), translated into words by a sensory-motor system controlling the phono-articulatory apparatus. The loss of language ability, as a result of injury, is an intolerable disability with, at present, very little chance of functional recovery. In order to design efficient rehabilitation actions, the knowledge of the functional organization of neural circuits that underlie language is mandatory.

The aim of PhD project was to investigate the functional properties of the cortical areas involved in sensory-motor control of speech by acting on the phono-articulatory apparatus. The phono-articulatory apparatus is innervated by the motor nuclei of the cranial nerves receiving, with few exceptions, bilateral input from the Primary Motor cortex (M1). At present, however, it remains unclear which other cortical areas in the frontal lobe are actually involved in shaping the motor program to be executed by M1 to allow verbal production and which is their precise functional role in sensory-motor control of the phono-articulatory gestures. Main focus of the project were the three frontal area classically considered involved in motor control of speech: Broca's area, vPM and M1.

A putative direct role of Broca's area in motor control of speech must be exerted either in shaping the activity of M1 or through its independent control of bulbar motoneurons. In both cases, Broca's area is expected to significantly affect, either directly or indirectly via M1, the motoneuronal excitability and, in turn, the activity of phono-articulatory muscles. However, the observation that injuries of sole Broca's area do not result in a motor deficit of speech, but rather in improving mutism (see Introduction), raised doubts on its significant role in control of speech motor output. Interestingly, the "apraxia of speech", a clear phono-articulatory dysfunction (see Introduction), follows to lesions of the ventral Pre-Motor cortex (vPM), suggesting to be involved in motor programming of speech. However, all these issues remained unresolved for many years, due to the lack of appropriate experimental tools to study these areas in humans, in ecological conditions.

In the last two decades, the introduction of the intraoperative *brain mapping* technique allowed a direct investigation of the functional properties of M1, vPM and Broca's area, adding new elements to the debate regarding the role of these areas in speech. However, the absence of a careful analysis of phono-articulatory activity during the brain mapping, highlights confounding evidence regarding the actual role of three areas in motor control of speech.

This study was performed by analysing data collected intraoperatively in 70 patients during surgical removal of gliomas performed with the aid of the *brain mapping* technique by a high skilled

neurosurgical team. The instrumental setup and the methodology used to perform the *brain mapping* technique gives the unique opportunity to investigate human circuits underlying language with a direct approach (see Introduction). During resection of gliomas located within or in proximity to the cortical areas and tracts involved in language neural network (at level of frontal, temporal or insula lobes), Broca's area, vPM and M1 are exposed and electrically stimulated as essential part of the clinical procedure. During the intraoperative phase, the patient is awakened and asked to perform different types of language tests assisted and evaluated online by neuropsychologists. During the language tests (object picture naming and counting tests), the Direct Electrical Stimulation (DES) is applied on the exposed areas (M1, vPM and Broca's area), in order to identify the eloquent cortical sites, i.e. the sites where DES actually "interferes" with the language function inducing a deficit in performance.

To perform a reliable mapping procedure, at the beginning of surgery the precise site onto the three areas expected to control the phono-articulatory apparatus and therefore to be likely involved in speech must be identified. It is indeed known in literature that both M1 and vPM host the representation of both oro-facial and hand-arm muscles (M1 controls also foot-leg muscles). Thus, during procedure first it is mandatory to identify, during each area the hand-arm and the oro-facial, the latter to be then exposed to stimulation during speech tasks. Once the oro-facial representation in the areas was disclosed, DES was applied on the areas during language tasks and the performance was compared with the performance of the same task without DES (*natural performance*). During all the surgical procedure, the electrical activity (EMG) of some of the muscles involved in the phono-articulation has been recorded. The EMG signal was analysed offline with a quantitative approach allowing to investigate the pattern of motor units voluntarily recruited during the language tasks performed by the patient in absence of stimulation (*natural performance*) to be then compared to the pattern of recruitment recorded when DES was applied on the three different cortical areas in the same conditions. This analysis was designed to disclose the specific pattern of specific alteration in motor unit recruitment in phono-articulatory muscles due to the DES-induced "transient inactivation" of the three areas. These data, interpreted in light of the animal studies and human studies, were used to infer the putative specific role of M1, vPM and Broca's area in motor control of phono-articulatory muscles in the attempt to shed light on the cortical network underlying the executive branch of language network.

2. *Materials and Methods*

The primary aim of the study was to investigate the functional involvement of Broca's, ventral pre-motor (vPM) and primary motor (M1) cortices in motor control of speech. The study was performed in patients affected by gliomas during the surgical resection performed with the aid of the *brain mapping* technique.

During the surgery of some gliomas harbouring within or in proximity to the cortical areas, and fibre tracts associated with language, the three areas were exposed under general anaesthesia, according to clinical needs, then the patients were awakened and asked to perform two different of speech tasks. During the tasks performance, the Direct Electrical Stimulation (DES) was applied onto the exposed areas in order to identify the eloquent cortical sites, i.e. where DES induced a transient impairment of the task execution due to its interference with the physiological activity of the stimulated area. At the beginning of surgery, the precise site onto the three areas expected to control the phono-articulatory apparatus was identified. DES was applied on the oro-facial sites during task performance and the eloquent site identified. Task performance was monitored in two conditions, i.e. during stimulation and in absence of stimulation. The activity of phono-articulatory muscles was recorded in both conditions to be compared with a quantitative analysis of the EMG signal performed offline.

Oro-facial representation in the three areas. To this aim, DES has been applied on three areas in two conditions: in muscle resting state and in a muscle pre-activated state. In the first condition, the muscular activity was at rest (background condition), while in the second condition, the oro-facial muscles were pre-contracted. The occurrence of movements, during DES application, was monitored in real time by an EMG system, allowing to identify the oro-facial representation on the different areas.

Mapping the DES interference-effects on task performance in the three areas. Once the oro-facial representations have been identified, DES was applied onto three areas while patients performed two speech tasks: counting and object picture naming. The position of each cortical-stimulated site has been registered intraoperatively into the neuro-navigation system to design, offline, a map where to localize, onto the different cortical areas, the different types of responses. From some available stimulation points have been also possible to reconstruct the probabilistic map of terminations, within

of the frontal lobe, of the main systems of fibers sub-serving the language network, calculated with an advanced tractography technique (HARDI, High Angular Resolution Diffusion).

Analysis of performance. During all the surgical procedure, the electrical activity (Electromyography: EMG) of some of the muscles involved in the phono-articulation (routinely monitored in clinical procedure) has been recorded. By recording the muscular activity, it has been possible to analyse the EMG signal offline and to investigate, by means of quantitative approach (by means of root mean square and of power spectrum) the pattern of motor units voluntarily recruited during the language tasks performed by the patient in absence of stimulation (*natural performance*). Once the specific pattern of motor unit recruitment during *natural performance* has been characterized, it was compared with the EMG activity of the same muscles when DES was applied onto the different cortical areas during the same language tests, in order to disclose the specific alterations in muscular activity occurring during interference on the three areas. The selected and analysed muscles have been the *orbicularis oris*, the *mentalis*, the *mylohyoid* contra- and ipsilateral muscles to stimulated hemisphere, and sometimes the *platysma* contralateral muscle (**Fig. 1.39**). The *orbicularis oris* and the *mentalis* are responsible of lips closure and lengthening of vocal tract, while the *mylohyoid* and the *platysma* of lips opening and shortening of vocal tract.

In an ideal experimental setting, this investigation should be performed by comparing the EMG activation in *natural performance* with the EMG activation impaired by DES, applied on M1, vPM and Broca's area, when the patient denominates the same word. However, the clinical constraints do not allow this procedure: for clinical needs, the patients denominate different words during the tasks, therefore it was not possible to compare the EMG activity corresponding to the same word during *DES-interference* and *natural performance*. In order to overcome this limitation, a preliminary study has been performed in a healthy subjects group (N=10) aimed at investigating whether the pattern of EMG activation (i.e. motor unit recruitment) exerted by the phono-articulatory muscles to articulate different words is comparable. It was mandatory to verify this condition to perform the comparison with the EMG activation during DES stimulation over the different cortical areas in patients denominating different words.

2.1 PATIENTS

In this study, 70 patients affected by gliomas were selected. Patients were underwent to a neurological and neuropsychological evaluation to characterize possible neurological and cognitive deficits, affecting the motor and/or language functions.

Patients were included in this study based on the following criteria:

- localization of the tumour in the dominant language hemisphere (the left hemisphere in 100% of patients);
- localization in the frontal, temporal or insular lobes;
- lack of tumour infiltration and reorganization of Broca's area, vPM and M1 at the time of investigation (Fornia et al., 2016);
- physiological phono-articulatory function.

We collected data concerning the application of DES to Broca's area in 27 patients (out of 70), to vPM in 58 patients and to M1 in 31 patients, since, in 70 patients considered, not all three areas were exposed in parallel during the surgical removal of tumours.

The patients gave written informed consent to the surgical and mapping procedure, which followed principles outlined in "World Medical Association Declaration of Helsinki: Research involving human subjects". The study was performed following the routine procedure adopted for surgical tumour removal, without introducing any variation.

2.2 PRE-OPERATIVE ROUTINE

All patients were submitted pre-operatively to neuroradiological, neurological and neuropsychological evaluation (Bello et al., 2007) to detect deficits due to the tumour.

Neuroradiological investigation:

In the pre-operative neuroradiological evaluation were performed:

1) the basal and volumetric Magnetic Resonance (MR, including T1, T2, FLAIR (Fluid-Attenuated-Inversion-Recovery), DWI and post contrast T1 images) were used to identify the tumour location, volume and its relationship with the surrounding structures (Bello et al., 2014).

2) the fMRI (functional Magnetic Resonance Imaging) using functional tasks (see below) to identify the most relevant cortical areas of the frontal lobe: M1, the Supplementary Motor Area (SMA), vPM and Broca's area. The hemispheric language dominance was determined by the laterality index, based on the fMRI results in language tasks.

3) the tractography analysis, performed by means of DTI-FT (Diffusion Tensor Imaging with Fiber Tractography) and, in some patients, by HARDI (High Angular Resolution Diffusion Imaging) techniques, to identify the most relevant systems of fibers, i.e. the M1 cortico-descendent fibers and

the most relevant subcortical pathways sub-serving language function: the inferior frontal-occipital *fasciculus*, the inferior longitudinal *fasciculus*, the superior longitudinal *fasciculus*, the arcuate *fasciculus*, the frontal aslant tract and the uncinate *fasciculus* (Caverzasi et al., 2016). The fibers reconstructions were upload on the neuro-navigation system to allow the intraoperatory identification of the fibers, running around or inside the tumour, and the anatomical and functional boundaries of the lesion. Neuroradiological analysis was performed by a neuroradiologist.

1) Volumetric RMI: anatomical tumour localization

Volumetric scan analysis was used to define the tumour location and volume (Bello et al., 2007). In all the patients, images were acquired in T1 and T2-weighted sequences with and without contrast administration; furthermore, FLAIR MRI sequences were acquired in all patients. Tumour volume was computed on volumetric FLAIR MRI scans for low grade gliomas and on post contrast T1-weighted MRI scans for high grade gliomas (Bello et al., 2007; 2014).

MR imaging were acquired using a 3T scanner (Philips Intera, Best). Standard MR evaluation for morphological characterization of lesions included axial T2-weighted TSE sequence (TR/TE 3000/85 milliseconds; field of view (FOV), 230 mm; 22 slices; section thickness, 5/1-mm gap; matrix, 512×512; SENSE factor, 1.5), axial 3D-FLAIR sequence (TR/TE 10 000/110 milliseconds; FOV, 230 mm; 120 slices; section thickness, 1.5/0-mm gap; matrix, 224×256; SENSE factor, 2) and postcontrast T1-weighted inversion recovery sequence (TR/TE 2000/10 milliseconds; FOV, 230 mm; 22 slices; section thickness, 5/1-mm gap; matrix, 400×512; SENSE factor, 1.5).

2) fMRI: functional identification of cortical areas

fMRI allowed the detection of cortical areas significantly activated during the tasks performed in the scan (sensory-motor or cognitive) with respect to other areas not significantly activated, thus identifying the areas involved in the task execution and hence part of the neural network underlying to the task execution. The patients enrolled in this study were asked to perform, during the image acquisition period, tasks activating selectively M1 (e.g. finger tapping task), SMA (e.g. bimanual finger tapping task), the ventral-lateral portion of vPM (e.g. mirror task: observation and execution of grasping movements, see Cerri et al., 2015) and language cortical areas, as Broca's area and vPM (e.g. covert object picture naming, fluency, covert auditory verb generation tasks) (Ruge et al., 1999). The acquired images were then analysed with a GLM-type model (General Linear Model) using MatLab, corrected for movement artefacts and aligned. Anatomical and functional images were subsequently normalized according to the MNI model.

fMRI images were acquired with T2-weighted sequences ((TR=3000 ms; TE=30 ms; flip angle=85°; FOV=240 mm; matrix=128x128; number of slices=40; thickness 3 m; SENSE factor AP=2) by means of the BOLD (blood oxygenation level-dependent) technique.

3) Tractography: identification of the subcortical pathways

DTI-FT

The Fiber Tracking (FT) with the DTI model of tissue water displacements assumes that the direction of least restriction as estimated by principal eigenvector of the fitted tensor corresponds to the direction of white matter tracts (Bucci et al., 2013). This DTI model is the ideal technique for describing a single fibre population within a given voxel. In these patients, DTI allowed the reconstruction of the fibers running around and through the tumour, their visualization and identification of the anatomical boundaries of the lesion. To reconstruct the component of the cortico-descendent tract originating from M1, a region of interest was placed on an axial section at the level of subcortical white matter of the pre-central *gyrus;* eventual contaminating fibers were removed. The same method was applied to reconstruct the subcortical pathways sub-serving language.

DTI data were obtained at 3T using a single-shot echo planar imaging (EPI) sequence (TR/TE 8986/80 milliseconds; b-value ¼ 1 000 s/mm2; 32 diffusion gradients directions; FOV, 240; isotropic acquisition voxel dimensions, 2.5×2.5×2.5 mm; acquisition matrix, 96×96; 56 slices, no gap; SENSE factor, 2.5).

HARDI

In juxtaposition to DTI, HARDI provides data about the orientation distribution function that can be used to determine the orientations of more fibers populations contributing to a voxel's diffusion MR signal (Bucci et al., 2013). For this reason, in a subgroup patients (6 out of 70, see below), the HARDI sequence was acquired in order to reconstruct, in post-operative phase, the extremity of principal fibers of language network, to define, with higher probability how cortical areas were connected by white matter fibers (see post-operative analysis).

HARDI images were obtained on a 3T scanner (3T Achieva, Philips Healthcare) using a diffusion-weighted spin echo EPI single-shot pulse sequence with the following parameters: 60 diffusion gradient directions, b-value=3000 s/mm2, TR/TE = 12000/74 ms, SENSE factor = 2, in-plane resolution = 1.87x1.87 mm2; thickness = 2.5 mm; no gap, FOV = 240 mm2, acquisition matrix = 128 × 128, data averages = 1, total scan time 13 minutes. A 3D T1-weighted sequence and a 3D-FLAIR sequence were also acquired for anatomical characterization. HARDI datasets were corrected for movement and eddy-current distortions using FMRIB Software Library (FSL). Diffusion Imaging in

Python (Dipy) software was used to estimate fractional anisotropy (FA) and for q-ball residual-bootstrap fibre tracking of language pathways (Caverzasi et al., 2014, 2016). Tracking was performed using an FA threshold = 0.1 and max angle = 60° as stopping parameters in the algorithm. The main white matter bundles belonging to language pathways and having terminations in the frontal lobe (arcuate *fasciculus* [AF], superior longitudinal *fasciculus* component II [SLF-II], SLF component III [SLF-III], SLF component temporal-parietal [SLF-tp], inferior frontal-occipital *fasciculus* [IFOF], uncinate *fasciculus* [UF] and frontal aslant tract [FAT]) were reconstructed using a 2- or 3-ROI approach, depending on the bundle (Caverzasi et al., 2016). Results were visualized using Trackvis (http://trackvis.org). Specifically, to reconstruct the IFOF and UF a single-plane seed ROI was defined on the FA colour map in the coronal plane passing through the anterior commissure, by selecting the anterior part of the left external and extreme capsules, where the two tracts run in contiguity. Target ROIs for the UF and IFOF were localized at the levels of the temporal and occipital lobes, respectively. For both tracts, the left frontal lobe was used as a second target ROI. Streamlines that passed through both target ROIs were retained. To reconstruct SLF-II and -III and AF a seed ROI was positioned in the coronal plane at the level of a region of high anteroposterior anisotropy lateral to the central part of the lateral ventricle and the corona radiata. Target ROIs were selected as follows: in the angular *gyrus* for SLF-II, in the supramarginal *gyrus* for SLF-III, and on the axial peritrigonal plane at the level of the posterior middle and superior temporal *gyri* for the AF. The left frontal lobe was used as a second target ROI. Streamlines that passed through both target ROIs were retained. To reconstruct FAT, the first region of interest was located in the white matter of the inferior frontal *gyrus* and the second region of interest in the white matter of the superior frontal *gyrus*, including the anterior cingulate and pre-supplementary motor area (Catani et al., 2013).

Neuropsychological investigation:

All patients were submitted to an extensive evaluation of the cognitive functions (language, memory, attention, apraxia, visuospatial abilities and executive functions) to detect specific deficits due to the tumour.

This project focuses on language and speech in particular, therefore only the assessment of these specific function will be reported. The language assessment was aimed at evaluating the spoken language, the level of education, the language production, comprehension and repetition. The pre-operative language assessment was also aimed at selecting and training the correct items to be used

in the “object picture naming task” during the intraoperative phase of surgery (see next paragraph, 2.3)

Language assessment

Patients underwent the following tasks (Bello et al., 2007):

- *oral controlled association by phonetic cue*: the patients are asked to name as many words as possible starting with the letters suggested by the examiner (e.g. F, P and L) in 1 minute (cut-off: at least 17 words per letter in the given time);
- *oral controlled association by semantic category*: similar to the previous test but, instead of letters, patients are given semantic cues (e.g. automakers, fruits and animals) (cut-off: at least 25);
- *object picture naming*: the patient is presented with 80 pictures: 30 inanimate objects (10 tools, 10 vehicles, 10 pieces of furniture), 30 animate objects (10 animals, 10 fruits, 10 vegetables), 10 pictures of body parts and 10 musical instruments (cut-off: 83% for inanimate objects and 87% for animate ones). This task was used during intraoperatively;
- *action picture naming*: the patient is asked to name the verb represented in a drawing (this is the verb oral naming subtest from the Batteria per l’Analisi dei Deficit Afasici, BADA);
- *word comprehension*: the word comprehension test includes 80 common nouns belonging to eight different categories (fruits, vegetables, animals, furniture, vehicles, tools, body parts, and musical instruments). For each stimulus, five pictures, corresponding to the target and four foils, respectively, are arranged vertically in a column on a card. The foils belong to the same category; therefore, only semantically associated errors are possible. For example, if the target word is “strawberry,” the other four words denoting fruits were presented together with the correct one. A stimulus word is read aloud by the examiner. The task of the patient is to choose the picture corresponding to the target. Sixty controls reached a score of 77 (97%) out of 80 correct answers, with a range of 72 to 80;
- *sentence comprehension*: this test included 80 items. The patients were shown a pair of pictures while one sentence was read to them. One of the pictures is correctly described by the sentence; the other corresponds to a sentence that is identical except for a detail (i.e., the boy is pushing the girl versus the boy is pushed by the girl). Given that no control subject scored <70 on this task in the original study, the score of 70 was taken as cut-off;
- *transcoding tasks*: all of the repetition subtests from BADA, including no-word repetition (35 stimuli), word repetition (36 stimuli), and sentence and syntagm repetition (20 stimuli) were performed. No errors are expected (no errors occur in controls).

- *token test*: comprehension of 36 oral commands divided into 6 groups of growing complexity, cut-off: 29;
- *counting*: counting from 1 to 10. This task was used during intraoperatively.

Table 2.1 reports the scores obtained by patients enrolled in the study in pre-operative evaluation of language abilities. Notably no pre-operative phono-articulatory deficits were found in our cohort. Some language deficits were found in patients, but they were not considered exclusion criteria for the investigation of the motor control of speech production. In the intraoperative phase, in fact, patients performed only two tasks: object picture naming and counting tasks and only items correctly named by the patient during the pre-operatory tests were used in the intraoperatory phase.

Table 2.1:

Patients	Token	W-C	S-C	O-N	A-N	S-F	P-F	W-R	NW-R	S-R	Phono-articulation
1	4	4	4	4	4	4	4	4	4	4	no deficit
2	2	4	4	4	4	3	4	4	4	4	no deficit
3	4	4	4	4	4	4	1	4	4	4	no deficit
4	4	4	4	4	4	4	1	4	4	4	no deficit
5	4	4	4	1	1	4	4	4	4	4	no deficit
6	4	4	4	4	4	4	4	4	4	4	no deficit
7	3	4	4	3	3	3	4	3	3	3	no deficit
8	4	4	4	4	4	4	4	4	4	4	no deficit
9	4	4	4	4	4	1	0	4	4	4	no deficit
10	4	4	4	4	4	4	4	4	4	4	no deficit
11	3	4	4	3	2	2	1	3	3	3	no deficit
12	4	4	4	4	4	4	4	4	4	4	no deficit
13	3	3	3	3	3	3	3	3	3	3	no deficit
14	4	4	4	4	4	4	4	4	4	4	no deficit
15	0	0	0	0	0	0	0	3	3	0	no deficit
16	3	3	3	4	4	4	4	0	0	0	no deficit
17	1	4	4	4	4	4	4	4	4	4	no deficit
18	3	4	4	2	0	1	2	4	4	4	no deficit
19	3	3	3	3	3	1	1	3	3	3	no deficit
20	3	3	3	4	4	3	3	3	3	3	no deficit

21	4	4	4	4	4	4	4	4	4	4	no deficit
22	3	4	3	3	4	4	4	3	3	3	no deficit
23	4	4	4	4	4	4	4	4	4	4	no deficit
24	4	4	4	4	4	4	4	4	4	4	no deficit
25	4	4	4	4	4	4	4	4	4	4	no deficit
26	4	4	0	4	4	1	0	4	4	4	no deficit
27	4	4	4	2	4	4	4	3	3	3	no deficit
28	4	4	4	0	1	0	1	4	4	4	no deficit
29	4	4	4	4	4	4	4	4	4	4	no deficit
30	2	4	3	2	3	4	0	4	4	4	no deficit
31	4	4	3	2	1	4	3	4	4	4	no deficit
32	4	4	4	4	4	0	1	4	4	4	no deficit
33	4	4	4	4	4	4	4	4	4	4	no deficit
34	4	4	4	1	1	4	4	4	4	4	no deficit
35	4	4	4	4	4	4	4	4	4	4	no deficit
36	3	4	3	2	4	3	4	4	4	4	no deficit
37	0	4	4	2	3	0	0	3	3	0	no deficit
38	4	4	4	0	3	0	2	4	4	4	no deficit
39	0	0	0	0	0	1	0	4	4	4	no deficit
40	3	3	3	0	3	0	3	3	3	3	no deficit
41	4	4	4	4	4	4	4	4	4	4	no deficit
42	4	4	4	0	0	0	0	4	4	4	no deficit
43	4	4	4	4	4	4	4	4	4	4	no deficit
44	4	4	4	4	4	4	4	4	4	4	no deficit
45	3	4	3	3	3	3	4	4	4	4	no deficit
46	3	3	3	3	3	3	1	3	3	3	no deficit
47	4	4	4	1	4	4	4	4	4	4	no deficit
48	3	3	3	3	3	3	3	3	3	3	no deficit
49	4	4	4	4	4	3	4	4	4	4	no deficit
50	4	4	4	2	0	4	4	4	4	4	no deficit
51	2	3	3	4	4	4	3	3	3	3	no deficit
52	3	3	3	3	4	4	2	3	3	3	no deficit
53	3	3	3	4	3	4	4	3	3	3	no deficit
54	3	3	3	3	3	1	1	3	3	3	no deficit

55	3	4	3	2	3	3	3	3	3	3	no deficit
56	3	3	3	3	3	3	3	3	3	3	no deficit
57	3	3	3	3	3	3	3	3	3	3	no deficit
58	3	3	3	4	4	3	1	3	3	3	no deficit
59	3	3	3	4	4	3	3	4	4	4	no deficit
60	3	3	3	3	3	3	3	3	3	3	no deficit
61	3	3	3	2	2	1	0	3	3	3	no deficit
62	3	3	3	1	1	1	1	3	3	3	no deficit
63	3	3	3	3	3	3	3	3	3	3	no deficit
64	3	3	3	3	3	3	1	3	3	3	no deficit
65	3	3	3	3	3	3	3	3	3	3	no deficit
66	3	3	3	3	3	3	1	3	3	3	no deficit
67	3	3	3	0	1	3	3	3	3	3	no deficit
68	3	3	3	3	1	3	3	3	3	3	no deficit
69	3	3	3	3	3	3	3	3	3	3	no deficit
70	3	3	3	3	3	3	1	3	3	3	no deficit

Table 2.1 Pre-operative evaluation of language abilities. Each task is evaluated with a score between 4 and 0. The value 0 indicates that the patient has a deficit in language-evaluated ability, 1 indicates a borderline condition between deficit and normality. The score between 4 and 2 indicates nothing language deficit, where 4 indicates that the patient does not perform nothing language error. In red are only highlighted the pathological conditions. Notably, in the last column is reported the absence of phono-articulatory deficits in each patient.

W-C = Word Comprehension; S-C = Sentence Comprehension; O-N = Object picture Naming; A-N = Action picture Naming; S-F = oral controlled association by semantic category or Semantic Fluency; P-F = oral controlled association by phonetic cue or Phonological Fluency; W-R = Word Repetition; NW-R = Not Word Repetition; S-R = Sentence and Syntagm Repetition.

2.3 SURGICAL PROCEDURE AND INTRAOPERATIVE ROUTINE

The intraoperative protocol included asleep-awake-asleep anaesthesia, the functional brain mapping and monitoring (*brain mapping* technique) and the neuropsychological assessment. In each patient, a craniotomy was tailored to expose the cortex corresponding to the tumour localization and a limited amount of surrounding tissue.

Anaesthesia

Total intravenous anaesthesia with propofol and remifentanil was used, no muscle relaxants were administered to allow mapping of motor responses. In order to prevent the occurrence of intraoperative seizures, the ECoG and free-running EMG were closely monitored. At the first ictal sign, the stimulation was immediately halted and cold irrigation was applied, which was usually sufficient to abort the seizure. Whenever seizures spread to the whole hemi body, propofol bolus infusion (4 mL on average) was delivered.

Neurophysiological brain monitoring

The monitoring included continuous ElectroCorticoGraphy (ECoG, ISIS-IOM, Inomed), a continuous ElectroEncephaloGraphic recording (EEG, ISIS-IOM, Inomed), a multichannel polygraphic recording of free-running ElectroMyoGraphic (EMG) activity and of the Motor Evoked Potentials (MEPs monitoring) (ISIS-IOM, InomedGmbH).

ECoG and EEG

ECoG, from a cortical region adjacent the area to be stimulated, was recorded by subdural strip electrodes (4-8 contacts, monopolar array referred to a midfrontal electrode; Cortical Strip Electrode, Integra LifeScience **Fig. 2.1**) through the whole procedure, to monitor the basal electrical activity of the brain and to detect after-discharges or electrical seizures during the resection. EEG, instead, was recorded with electrodes placed over the scalp in a standard array. EEG and ECoG signals were filtered (bandpass 1-100 Hz), displayed with high sensitivity (50-150 μV/cm and 300-500 μV/cm respectively) and recorded.

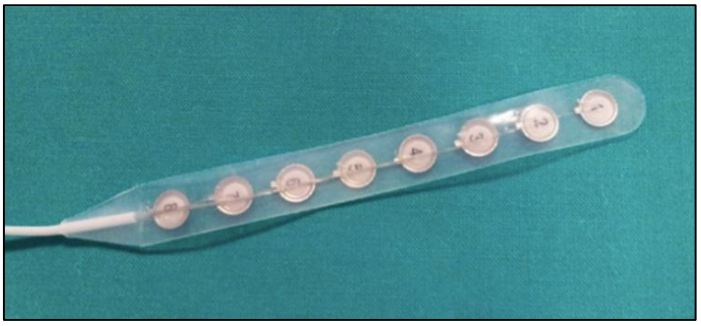

Fig. 2.1 silicon strip used for ECoG and MEPs monitoring. Integra LifeScience, Plainsboro, NJ.

MEPs monitoring

During brain surgery, the occurrence of lesions affecting the corticospinal tract originating from M1 must be avoided, leading in permanent deficits of the voluntary movement, resulting in severe disability and poor quality of life for affected patients. To preserve the integrity of these fibers, the descending motor pathways was monitored throughout the procedure using a "train-of-five" (To5) stimuli delivered to M1 cortex to elicit MEPs from face, upper and/or lower limb muscles (Taniguchi et al., 1993; Szelenyi et al., 2010), but was suspended during cortical and subcortical mapping to avoid interference with it. To this aim, a 4-8 contacts subdural strip electrode (**Fig. 2.1**) was placed over the pre-central *gyrus*. Each contact was tested, with a vertex reference, by stimulation with trains of 3-5 constant current anodal pulses (pulse duration: (0.5-0.8) ms; Inter-Stimulus Interval within the train (ISI): (3-4) ms) at a repetition rate of 1 Hz. The Motor Threshold (MT) corresponded to the lowest intensity allowing a reproducible MEP (peak-to-peak amplitude > 20 μV) in a muscle. The muscle activity of the patient was monitored during surgery (Bello et al., 2014) and it was recorded by pairs of subdermal hook needle electrodes (Technomed) placed in a muscle-tendon array. The target muscles for monitoring were chosen according to cortical exposure; typically, in face: *orbicularis oris* (OO), *mentalis* (MENT), *mylohyoid* (MYLO); in upper limb: *extensor digitorum communis* (EDC), *abductor digiti minimi* (ADM), *first dorsal interosseous* (FDI), *abductor pollicis brevis* (APB); in lower limb: *tibialis anterior* (TA), *triceps surae* (TRIC-SU), *flexor hallucis brevis* (FHB).

EMG recording

The muscle activity was also monitored since the very beginning of surgery, by multiple EMG recording: 20 muscles (face, upper and lower limb) contralateral to the hemisphere to be stimulated, plus 4 ipsilateral muscles connected to a multichannel ElectroMyoGraphic (EMG) recording (ISIS, INOMED, sampling rate 20 kHz, notch filter at 50 Hz). EMG was used to record responses to stimulation, the voluntary motor activity and to distinguish between electrical and clinical seizures. Close attention was paid to prevent intraoperative seizures by monitoring the ECoG and EMG: at the first ictal sign, the stimulation was stopped and cold irrigation was applied, to abort the seizure. Whenever the seizures spread to the whole hemi body, propofol bolus infusion (4 ml on average) was delivered.

Particularly important for this study, focused on the phono-articulatory apparatus, were the EMG recordings of some phono-articulatory muscles routinely monitored in clinical procedure and essential for the articulation (see Introduction): contra- and ipsilateral OO muscle, ipsi- and

contralateral MENT muscle, contra- and ipsilateral MYLO muscle and, when available, contralateral *platysma* muscle (**Fig. 2.2**).

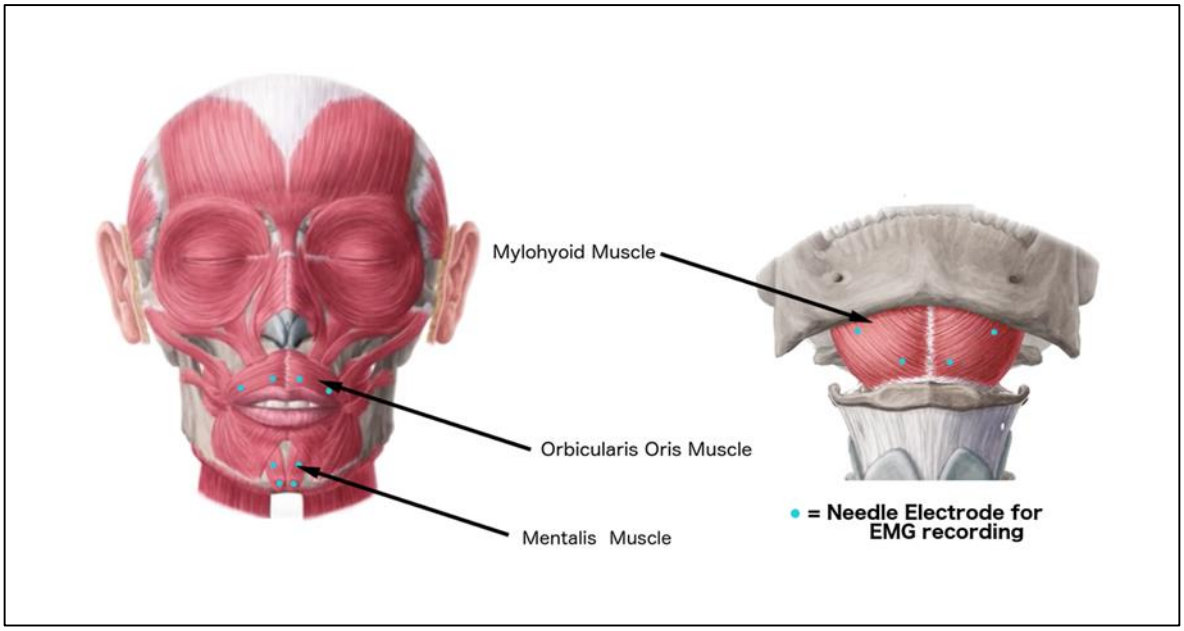

Fig. 2.2 Drawing of the phono-articulatory recorded muscles and analyzed off line. The pairs of blue dots represent the subdermal electrodes.

Brain mapping technique

The *brain mapping* technique is a functional intraoperative approach consisting in delivering Direct Electrical Stimulation (DES) on cortical and subcortical brain structures in asleep patients or in awake patients performing a behavioural task and it is expected to interfere with the execution of the task only when the current is applied onto structures (defined *eloquent* areas) belonging to the neural circuit sub-serving the execution of that specific task. In this study, all the patients were awake and the motor and/or language pathways were investigated by means of two stimulation paradigms: the Low Frequency (LF) and the High Frequency (HF) paradigms (Bello et al., 2104).

LF stimulation

The LF stimulation (or LF-DES) consisted in trains -lasting 1 to 4 sec- of biphasic square wave pulses (0.5 ms each phase) at 60 Hz (ISI=16.6 ms) delivered by a constant current stimulator (OSIRIS-NeuroStimulator) integrated into the ISIS-System through a bipolar probe (2 ball tips, 2 mm diameter, spaced by 5 mm, **Fig. 2.3**).

During the cortical mapping the intensity of LF paradigm, for the first stimulation trial, was set to an initial intensity of 2 mA, then increased in a stepwise fashion by 0.5 mA until it was possible to evoke a clear muscle response on the free-running EMG (cortical Low Frequency Motor Threshold - cLF MT). This protocol was repeated for each cortical site needed for mapping. The subcortical mapping will not be discussed here given that is not the main focus of this project.

HF stimulation

The HF stimulation (or HF-DES) was delivered through a monopolar probe (straight tip, 2mm diameter, Inomed with reference/ground on the skull in correspondence of the central sulcus, **Fig. 2.3**), it consisted in trains from 1 to 5 constant anodic current pulses (pulse duration 0.5 ms; ISI=(3-4) ms) at variable intensity depending on the patient's cortical excitability.

During the cortical mapping, the current intensity was initially set at the same value of MT used for the MEPs monitoring and, during mapping, subsequently reduced until reaching the lowest intensity able to consistently elicit motor responses (MEP peak-to-peak amplitude > 20 μV, cortical High Frequency Motor Threshold - cHF MT). This protocol was repeated for each cortical site needed for mapping. The subcortical mapping will not be discussed here given that is not the main focus of this project.

Probes

The probe was selected based on the stimulation protocol: HF-DES was performed with a monopolar probe, while LF-DES with a bipolar one (**Fig. 2.3**). It has been demonstrated that the electrical fields induced by the two probes, have different shapes: the electrical field induced by the monopolar probe spreads to a larger cortical area (**Fig.2.3** right), while the electrical field of the bipolar probe, conversely, is more focused (Bello et al., 2014). In some instances it is though possible the use of a bipolar probe with the HF protocol to obtain a more localized stimulation and a more precise identification of functional margins of resection (Bello et al., 2014).

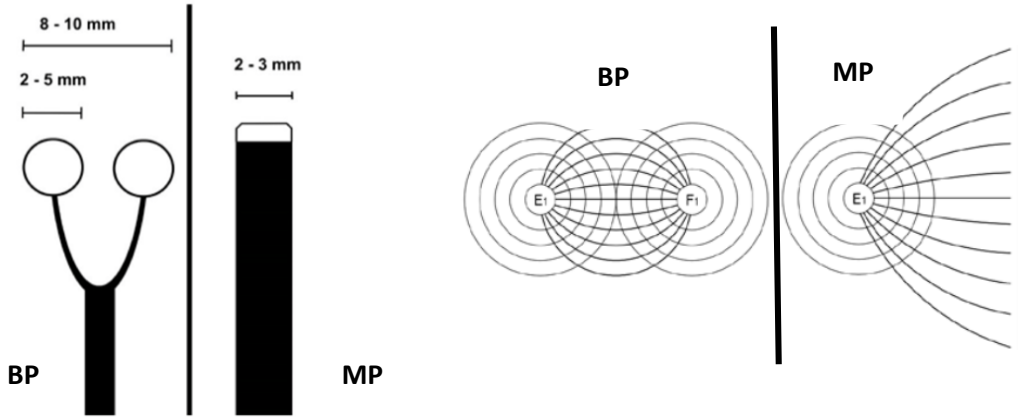

Fig. 2.3 Left: schematic of Bipolar (BP) and Monopolar (MP) Probes. Right: electric field distribution of the stimulations with BP and MP.

Intraoperative mapping of Broca's area, vPM and M1

Data collected for the project were related to the responses elicited in oro-facial muscles by cortical stimulation of Broca's area, vPM and M1. As defined in Introduction section, with different experimental approaches it has been demonstrated (Luders et al., 1987; Moor and Price, 1999; Van Turennout et al., 2000; Henry and Crawford, 2004; Hillis et al., 2004; Indefrey and Levelt 2004; Axelson et al., 2009; Ackermann and Riecker, 2010; Chang et al., 2011, 2015, 2016; Robinson et al., 2012; Tate et al., 2014, 2015; Flinker et al., 2015; New et al., 2015) that these areas are all involved in language. Regarding the motor properties of the same areas, it has been demonstrated, in animals and humans, that vPM and M1 are motor areas controlling hand-arm and oro-facial muscles, while the motor properties of Broca's area were significantly challenged in recent studies (Cerri et al., 2015). Consequently, the evidence of an oro-facial and/or hand-arm representation in Broca's area is lacking. Since the aim of the project was to investigate the functional role of Broca's area, vPM and M1 in motor control of speech by means of *brain mapping* technique, the identification of the precise site over vPM and M1 hosting the oro-facial representation (expected to control the phono-articulatory apparatus), by distinguishing it from the hand-arm representation, was performed by at the beginning of surgery: the two areas were stimulated with both LF-DES and HF-DES paradigm in resting or pre-activated muscle condition to elicit the motor responses recorded by subdermal electrodes inserted in oro-facial and hand-ram muscles. Despite in a previous study of our group, it was demonstrated that no motor output can be elicited by Broca's area stimulation, the same approach was used to explore Broca's area in the attempt of disclosing motor responses.

The complex clinical condition and the primary aim to avoid any impact on the clinical procedure time, did not allow collection of a full set of responses in all muscles from each areas in every patient: 27 patients were analysed for Broca's area, 58 for vPM and 31 for M1.

Identification of oro-facial and hand-arm representations on the three areas

The anatomic-functional identification of Broca's area, vPM and M1 were established by pre-operative study (fMRI and tractography, see above). For each patient, the fMRI and DTI (and in some cases HARDI) data were loaded on the neuro-navigation system to be available to the neurosurgeon during the procedure. Once three areas were exposed, they were stimulated with LF-DES and the HF-DES (taking into account fMRI results) to identify oro-facial and hand-arm responses.

LF-DES

The LF-DES was applied on Broca's area, vPM and M1, to detect motor responses by recording the activity, on free running EMG, of 24 contra- and ipsilateral muscles to the stimulated hemisphere (see above). The whole muscles activity was recorded for all duration of the LF-DES.

For this analysis, we have selected a subgroup of 5 patients: in all patients, the stimulus was applied onto Broca's area, vPM and M1. The LF stimulation was applied to M1 (average train duration ± SD: 1.7 ± 1.1 sec; average stimulation intensity ± SD: 3.2 ± 1.1 mA), onto the oro-facial and hand-arm motor areas in resting and in tonic pre-contracted states. The same protocol was applied to the vPM (average train duration ± SD: 2.1 ± 0.5 sec; average stimulation intensity ± SD: 3.6 ± 0.6 mA) and to Broca's area (average train duration ± SD: 2.3 ± 0.9 sec; average stimulation intensity ± SD: 3.9 ± 0.6 mA).

HF-DES

The HF-DES was applied was applied on Broca's area, vPM and M1 to detect elicited motor responses (MEPs) by recording the activity of six muscles contralateral to the stimulated hemisphere (always the left): two oro-facial muscles (OO and MYLO), four hand-arm muscles (EDC, APB, FDI and ADM). The whole muscles activity was recorded for all duration of the HF-DES.

For this analysis, we have selected a subgroup of 27 patients (out of 70). This number of patients is major in relation to LF protocol, because HF-DES can induce a minor number of electrical seizures. HF-DES was applied in resting state condition in 21 patients on vPM and M1, and in 10 on Broca's area, while, in pre-activated condition, HF-DES was applied in 10 patients on all areas. With the HF-DES protocol, 5 shocks were initially applied on M1 (average stimulation intensity ± SD: 15.4 ± 7.3 mA) at the threshold current intensity (or Motor Threshold, MT) necessary to induce a motor response

in oro-facial and hand-arm representations. Then, the current intensity was increased in order to obtain a response with a lower number of pulses (1 and maximum 3 shocks). The same protocol was applied to vPM (average stimulation intensity ± SD: 20 ± 9.4 mA) and to Broca's area (average stimulation intensity ± SD: 16.3 ± 5.3 mA). In this latter case, the number of shocks was increased to 7 shocks (see Results section).

DES-induced interference effects during task performance

Once three areas were exposed and the somatotopic representation of oro-facial muscles assessed, the LF-DES was applied with the bipolar probe on them, while the patients were performing two speech tasks, i.e. counting and object picture naming, based on the premises that when a brain area is involved in the ongoing task performance, DES interference should impair the *natural performance*. Mapping procedure during task performance started from the oro-facial representation, where available, then was extended to the cortex around the representation, to map all possible eloquent (i.e. responsive to DES) sites allowing a safer identification of the surgical point of entry (Bello et al., 2007).

During object picture naming task, the patient was asked to name the picture of an object presented on a computer screen. Pictures were presented with a regular timing allowing a pause of few seconds between subsequent pictures. As soon as the picture was presented, the patient was asked to name the picture, while during the pauses the patient was kept silent. During counting task, the count was self-paced, but patients were previously trained to wait few seconds between one number and the following one. During task performance (either counting or object picture naming), LF-DES was randomly applied on Broca's area, vPM and M1, so that some trials were performed without stimulation (*natural performance*) and other during stimulation (LF-DES *interference*).

The effect of stimulation (LF-DES *interference*) on the three areas during speech tasks, measured by clinical inspection, is well known (Matsuda et al., 2014; Tate et al., 2014; Chang et al., 2016, see Introduction) and, therefore, is part of the clinical routine in functional neurosurgical practice. As a standard routine, when the stimulation onto Broca's area stops ("speech arrest" phenomenon) the patient's counting/naming at least three non-consecutive times, the identification of Broca's area is considered reliable. Regarding vPM, when the stimulation induces disruption of speech referred as "anarthria" (a term so far considered a synonymous of speech arrest, Tate et al., 2014, 2015), at least three non-consecutive times, the identification of the area is considered reliable. Differently, the stimulation of M1 induces a disruption of speech with facial muscles contraction (Tate et al., 2014) or "dysarthria" (a motor impairment accompanied with dysphonic/aphonic speech; see Deletis et al., 2014).

According to the routine procedure for language mapping of our patients, the first areas to be identified were M1 and/or vPM. LF-DES paradigm was applied on M1 (on oro-facial representation) and/or vPM to identify the minimum current intensity (Threshold Intensity) needed to induce a clear interference (anarthria/speech arrest and/or dysarthria) in task performance (*ThreshI*-LF-DES). Then *ThreshI*-LF-DES was applied onto the putative Broca's area and the intensity of stimulation was increased until the "speech arrest" was obtained (*SupraThreshI*-LF-DES).

The phono-articulatory muscles recorded to analyse the motor performance were contra- and ipsilateral OO, contra- and ipsilateral MENT, contra- and ipsilateral MYLO and, in some cases, the contralateral PLATYSM (not always recorded). The activity of all the muscles was recorded for all duration of the LF-DES.

The analysis of the effect of DES delivered during the counting task was performed separately from that during object picture naming task.

For this analysis, 58 patients (out of 70) were considered: in 20 patients, the stimulus was applied on Broca's area, in 45 onto vPM, in 10 onto M1. Considering all the patients, the main parameters of LF-DES were:

i) M1: average train duration ± SD: 2.43±1,36 sec; average stimulation intensity ± SD: 3.00±1.15 mA;
ii) vPM: average train duration ± SD: 2.57±1.02 sec; average stimulation intensity ± SD : 2.65±0.83 mA;
iii) Broca's area: average train duration ± SD: 2,87±1,12 sec; average stimulation intensity ± SD: 3.64±1.37 mA.

Despite the HF-DES (trains of 5 shock delivered with a 3Hz train repetition rate) was recently demonstrated to be of comparable efficacy with respect to LF-DES in intraoperative mapping of language function (see Riva et al 2016). Our study, instead, was performed on data collected by using LF-DES paradigm due to technical reasons: the LF-DES stimulus artefact was easily filtered out from the EMG of phono-articulatory muscles (see below), while it is not possible to filter the HF-DES stimulus artefact. Moreover, all the neurosurgical literature available at present reports data and observations collected by stimulating with the classical LF-DES protocol therefore, to allow to safely refer to previous studies performed in the same setting, we decided to select data collected with the same methodology.

2.4 POST-OPERATIVE ANALYSIS

Identification of oro-facial and hand-arm representations in M1, vPM and Broca's area

During the intraoperative procedure, the MEPs elicited by HF-DES were recorded with specific software (ISIS, INOMED, sampling rate 20 kHz, notch filter at 50 Hz). In each patient (N = 27), in order to perform the analysis of the motor responses, all the MEPs recorded during the procedure were extracted from the acquisition system and resampled at 4 kHz and analysed offline by means of dedicated software (MatLab, MathWorks 2009b). For each trial, a window of interest of 100 ms from the stimulus onset was defined. The average background EMG activity and its standard deviation (± 1SD) were then calculated from the last 25 ms of the record (i.e. from 75 to 100 ms). A MEP was considered reliable and consistent, if the EMG signal exceeded significantly the average background ± 1SD. If so, the MEPs onset latency, duration and amplitude was determined. Once extracted, the MEPs were stored based on type of muscle and area (M1 *vs* vPM *vs* Broca's area). We focused on responses of six muscles: two oro-facial muscles -OO and MYLO- and four hand-arm muscles -EDC, FDI, APB and ADM-, as defined above. Importantly, we considered only the MEPs obtained when the patients were fully awake during the procedure to be reliable. For the analysis of MEPs in resting condition, we considered only those recorded when patients were relaxed. MEPs occurring during voluntary muscle activation, e.g. due to postural adjustment, were not considered. Moreover, the offline analysis was performed with great care to identify MEPs possibly contaminated/facilitated by voluntary movements. This condition was detected by visual inspection of the raw EMG activity and all pre-activated MEPs were excluded from the analysis.

The parameter considered for the comparison between M1, vPM and Broca's area was the latency of MEPs.

Comparison of motor responses and DES-induced interference effects in M1, vPM and Broca's area

Analysis of MEPs latency: M1 vs vPM vs Broca's area

For each MEP in each analysed muscle, the absolute latency, (i.e. the delay between the effective stimulus and the MEP onset) was calculated. The intraoperative stimulation protocol included single stimuli and/or with trains of variable number of stimulus pulses (1 to 5 stimuli – 1 to 7 for Broca's area - with ISI of 3-4 ms), depending on the clinical need, conditions and purpose. Due to clinical

constraints, the surgeon did not introduce variations of the mapping procedure. When the responses were elicited with a single stimulus, the delay was easily computed between the stimulus artefact and the onset of the response. When a train of stimuli was applied, the effective stimulus (i.e. the stimulus actually inducing motoneurons discharge and, in turn, the motor response) was calculated by means of a probabilistic estimation applied at single subject level, using as a reference the latency of MEPs elicited with a single stimulus. The main assumption in this estimation was that, within a train of shocks, the latency measured from the stimulus (e.g. the third in a train of 5 shocks), which is supposed to be the effective one, and the MEP onset should be equal (within mean ± 1SD of M1 latency single pulse) or longer (more than mean ± 1SD of M1 latency single pulse) but never shorter (less than mean ± 1SD M1 MEP latency single pulse) with respect to the latency of a MEP evoked in the same site with a single stimulus. According to this assumption, when the responses were elicited with a train of stimuli, the effective stimulus within each train was identified/selected and, accordingly, the latency of the evoked response calculated. The same analysis was applied for each muscle with responses from M1, vPM and Broca's area. The stimulation of Broca's area always failed to elicit any response in any muscle (see Results section), even when increasing the number of shocks (up to 7). For this reason, we consider for following analysis only 21 patients (out of 27 in this section). In 50% of these subjects, responses to a single stimulus were available from both areas (vPM and M1). In all the other subjects, single stimulus was effective in eliciting responses from M1, while vPM was responsive to trains of stimuli. In this specific condition, the effective stimulus was calculated with the same procedure described above using as a reference the latency (mean ± 1SD) of MEPs evoked form M1 with 1 shock. Responses elicited from M1 were indeed expected to be the fastest, due to its direct connections with motoneurons (Cerri et al., 2003; Shimazu et al., 2004). Given these premises, the patients without positive responses to single stimuli from M1 were excluded from this study.

DES-induced interference effects during task performance

In order to disclose the effects on phono-articulatory activity induced by LF-DES application on Broca's area, vPM and M1 during speech tasks, the output of the performance (clinical inspection) was evaluated in the intraoperative phase by neuropsychologist and by online neurophysiological monitoring; successively, an offline analysis of the EMG of the phono-articulatory muscles recorded during the *natural performance* (object picture naming and counting in absence of DES) was compared with the EMG recorded in the same muscles (during the same task) when LF-DES was applied to Broca's area, vPM and M1, separately.

At the state of art, in the same clinical setting (Tate et al., 2014; Chang et al., 2016), the interferences induced by LF-DES are routinely characterized by clinical inspection (visual detection of movement and neurophsycological evaluation of errors) without EMG recording. However, the sole report of the impairment based on clinical inspection does not allow to precisely investigate the motor performance and the motor properties of three areas, therefore a quantitative analysis on the muscle activity was needed.

The quantitative analysis of the EMG signals, performed offline on intraoperatively-recorded EMG traces of some the phono-articulatory muscles (**Fig. 2.2**), allowed to investigate the recruitment of motor units during the speech task affected by LF-DES compared to *natural performance* of the task. If Broca's area, vPM and M1 were actually involved in motor control of phono-articulatory muscles during speech, the stimulation would induce a significant quantitative alteration of the most representative EMG parameters (see below) when compared with the EMG parameters of the *natural performance.* However, in an ideal experimental setting, this investigation should be performed by comparing the EMG activation in *natural performance* with the EMG activation impaired by DES, applied on M1, vPM and Broca's area, when the patient denominates the same word. The clinical constraints did not allow this procedure: for clinical needs, the patients denominated different words during the tasks, therefore it was not possible to compare the EMG activity corresponding to the same word during *DES-interference* and *natural performance*. In order to overcome this limitation, a preliminary study has been performed in a healthy subjects group (N=10) aimed at investigating whether the pattern of EMG activation (i.e. motor unit recruitment) exerted by the phono-articulatory muscles to articulate different words was comparable. This analysis was mandatory for the following analysis on EMG signals recorded in patients.

Healthy subject. The healthy selected-subjects (N=10) were not affected by language and motor disorders. They performed the same tests routinely adopted in the intraoperative phase by patients: object picture naming and counting (see above). In first test, they were asked to name the picture of an object presented on a computer screen. The name of the picture was earlier selected. Pictures were presented with a regular timing allowing a pause of three seconds between subsequent pictures. As soon as the picture was presented, the subject was asked to name the picture, while during the pauses the subject was silent. The number of pictures was 50. In last task, the subject was asked to count from 1 to 10, maintaining the same timing between two consecutive words (three seconds). The tasks were repeated 5 times ((50 pictures + 10 numbers) * 5 times). During tasks, the muscular activity of the OO, MYLO, MENT and PLATYSM *muscles* (left and right), the same recorded-muscles in

patients (Fig. 2.2), was recorded using by pairs of surface electrodes and then extracted by means of a script programmed in MatLab (version 7.9.0). To investigate whether the pattern of EMG activation exerted by the phono-articulatory muscles to articulate different words was comparable, we calculated for each recorded-muscles, for each denominated-words, the power spectrum (PS) of the EMG signals (see **BOX 1**). After that, each PS was normalized and compared two by two by calculating, for each comparison, the Correlation Coefficient (CC, see **BOX 2**). In collaboration with a statisticians group (Dr. Matteo Borrotti and Dr. Antonio Pievatolo) was created a more complex statistical model (called "Mixed-effects model", see below) to allow if in two different words compared for the representative-frequency in PS, for the same muscle, the two logarithmic frequency means was not significantly different and, thus, it was not be possible to distinguish between the two words.

Many common statistical models can be expressed as linear models that incorporate both fixed effects, which are parameters associated with an entire population or with certain repeatable levels of experimental factors, and random effects, which are associated with individual experimental units drawn at random from a population. A model with both fixed effects and random effects is called a mixed-effects model (Pinheiro and Douglas, 2000). Mixed-effects models are flexible tools for modelling the within-group correlation often present in grouped data. An example of grouped data includes the repeated measures (our instance). Furthermore, mixed-effects model handle balanced and unbalanced data in a unified framework.

For N independent sampling units (i.e. subjects), the linear mixed model for subject *i* may be written

$$yi = Xi\,\beta + Zi\,di + \varepsilon i$$

where, yi is a $pi \times 1$ vector of observations on subject i; Xi is a $pi \times q$ known, constant design matrix for subject i, while β is a $q \times 1$ vector of unknown, constant, population parameters (fixed effects). Also Zi is a $pi \times m$ known, constant design matrix for subject i corresponding to the $m \times 1$ vector of unknown random effects di, while εi is a $pi \times 1$ vector of unknown random errors (Edwards et al., 2008).

Diagnostics of a mixed model was done with ANOVA analysis, which was used to determine if two mixed models were different or not. ANOVA uses F-tests to statistically test the equality of model means and variances (Beckman et al., 1987).

Patients. For this analysis, we considered 58 patients, all stimulated over left hemisphere (dominant language hemisphere). The EMG signal of contra- and ipsilateral OO, MENT, MYLO muscles and some times of contralateral PLATYSM muscle was recorded intraoperatively (**Fig. 2.2**),

and then extracted by means of a script programmed in MatLab (version 7.9.0). Offline, three conditions were analysed:

i) the EMG signal recorded during the *natural performance* (object picture naming and/or counting);
ii) the EMG signal recorded during the resting state condition (or BackGround signal, BG-EMG);
iii) the EMG signal recorded during LF-DES applied on M1, vPM and Broca's area during speech tasks (object picture naming and/or counting).

In order to compare the EMG signal recorded in *natural performance* with that occurring during *LF-DES interference,* three subsequent analysis were performed:

1) at first the EMG signal corresponding to the muscle activation occurring during *natural performance* was selected from to the EMG signal corresponding the resting condition (background EMG);
2) the EMG signal corresponding to muscle activation occurring during *LF-DES interference* was selected using as a reference the stimulus artefact indicating the exact timing of the stimulation recorded in a dedicated EMG channel (*orbicularis oculi*);
3) the EMG signal corresponding to the muscle activation during the *natural performance* was compared with the EMG signal occurring during *LF-DES interference*.

1) *Natural performance*. The first analysis was aimed at selecting and extracting, for each phono-articulatory recorded muscle, the EMG signal corresponding to the muscle activation occurring during the *natural performance* (i.e. task-related EMG). To this aim, the EMG recorded signal from the phono-articulatory muscles was rectified and the Root-Mean-Square (RMS) was calculated across an epoch (time window) of 100 ms, with a sliding window of 50 ms to the preceding one. RMS background was calculated by averaging four part of EMG signal in resting condition selected randomly plus its 3*SD. This latter value was chosen to exclude from background signal non-specific muscular activity possibly occurring during small and non-task related movements, possibly creating a false positive EMG activation. For each trial, the onset and the offset of the task-related muscle activity were extracted by subtracting the RMS background activity from each trial of the task, i.e. by setting at the point of intersection between the 3*SD line and the RMS slope corresponding to the onset or offset of the EMG activation of the phono-articulatory muscle during tasks (**Fig. 2.4**). In **BOX 2**, we have estimated the pattern of activation of oro-facial muscles in *natural performance*.

2) *LF-DES interference*. The EMG signal corresponding to *LF-DES interference* was selected by using as a reference the stimulation artefact recorded by one of the EMG synchronized-channels

(*orbicularis oculi*). The selected signal corresponded to the onset and offset of the stimulation artefact. Overall a total number of 97 trials were recorded (mean number of trials for subject ± SD = 4.85 ± 3.79).

3) *Natural performance vs LF-DES interference.* When the EMG signal corresponding to all trials of *LF-DES interferences* onto Broca's area and *natural performances* were identified and selected, the two conditions were compared. The comparison was performed by using quantitative parameters of the EMG signal, calculated in time and frequency domains.

Time domain. The RMS was selected as the main parameter for the analysis of the EMG signal in time domain: the mean and peak values (in µV) RMS were compared in the two conditions.

Frequency domain. The power spectrum (PS) of the signal (Fast Fourier Transform) was computed to estimate the mean frequency, the area (in μV^2) under the spectrum curve and median frequency.

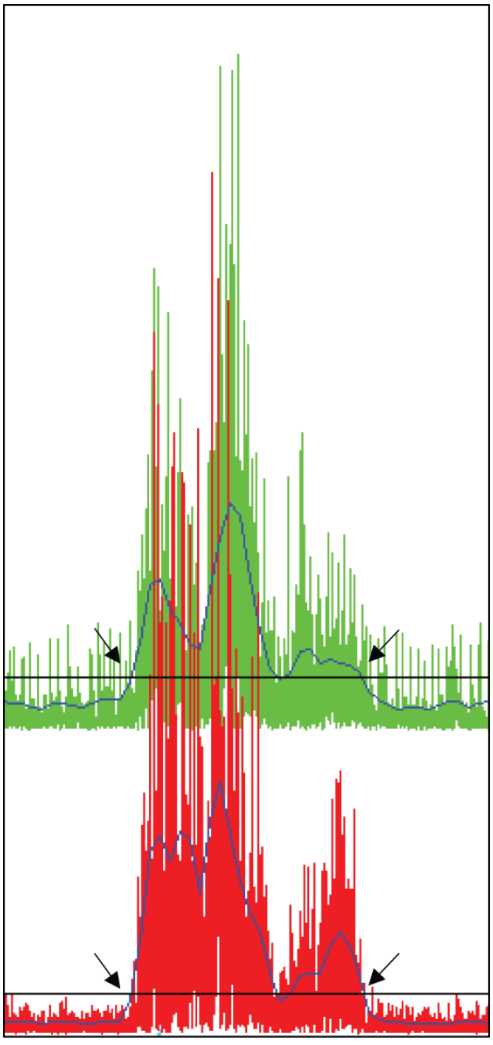

Fig. 2.4 Fragment of EMG signal corresponding to *natural* speech *performance*.

EMG signal related to *Orbicularis Oris* (OO) contra- (green trace) and ipsilateral (red trace) muscle. Both EMG signals are rectified. The horizontal line (black) correspond to the value of three times the standard deviation (3*SD) of the mean amplitude (in µV) of the background EMG, while the blue line on EMG signals correspond to root mean square (RMS)

The onset of the EMG activity related to natural performance was set at the point of intersection between the 3*SD line and the increase of RMS slope (arrows at left). Accordingly, the offset of the EMG activation during tasks was set at the point of intersection between the line and the decrease of RMS slope under the 3*SD line (arrows at right).

For each muscle analysed in either (object picture naming or counting) tasks, the comparison of the EMG activity between the *natural performance* and the *LF-DES interference* was therefore computed

on 5 parameters: the mean and peak values for RMS and the mean and median frequencies, and area under the curve for PS.

In **BOX 1** we have described with more accuracy the EMG parameters.

BOX 1. EMG parameters

The EMG signals, related to *natural* speech *performance* (object picture naming and counting tasks, in patients and in subjects), LF-DES *interference* and BG-EMG traces were analysed in time and in frequency domain.

Time domain: Root Mean Square

To analyse the signal in the time domain, we calculated the Root Mean Square (RMS), a more accurate index of the muscle activity compared to raw EMG signal (Firlund and Cacioppo, 1996), that reflects the mean power (μV) of the EMG signal. The algorithm to calculate RMS needs the definition of the EMG epoch (time window) to be computed; in this study, RMS values were calculated for time windows of 50 ms (2 KHz sampling frequency) and each window overlapped the previous and the next one for 20 ms. The mean and peak values (in μv) of RMS of each fragment of interest were calculated.

At physiological level, mean RMS value identifies changes occurring in recruitment of the motor units: the signal can be increased by increased recruitment of a given motor unit or recruitment of larger units.

The RMS was used in two conditions:

1) to pinpoint the onset and offset points of *natural* speech *performance* compared to BG-EMG signal, but with different time windows.
2) to allow as LF-DES can interfere with the natural speech performance, when applied on three different cortical areas, by comparing the EMG signals related to *LF-DES interference* and to *natural performance*.

Frequency domain: Power Spectrum

The EMG frequency power distribution was calculated by means of a mathematical algorithm, i.e. the "Fast Fourier Transformation" (FFT), and graphically presented as a total Power Spectrum (PS) of the EMG signal, which shows the frequency power distribution (Y-axis) in ratio to the frequency band (X-axis). The EMG frequency power corresponds to a frequency range of 10-250 Hz. Therefore, by means of FFT, it's possible to analyse and estimate the frequency contents of EMG signals. The main parameters selected were the mean frequency, the total power as the area (in μv2) under the spectrum curve and the median frequency.

At physiological level, PS parameters represent a measure of the frequencies of discharge of the motoneurons innervating the muscles. The PS area indicates which is the range of frequency of discharge of the motor units recruited during the task execution.

The activity of the phono-articulatory muscles in *natural performance* (object picture naming and counting tasks, separately) was compared to the activity of the same muscles when the LF-DES was applied on M1, vPM and Broca's area individually. Comparison was computed for each word denominated in the two tasks (counting and object picture naming) and in the two conditions (*LF-DES interference* and *natural performance*), on five EMG parameters of each recorded muscles: 1)

mean and peak values for RMS, 2) mean and median frequencies and area under the curve for PS. Statistical analysis was computed with Statistica 7.1 software and described separately.

Statistical analysis

Analysis of MEPs latency: M1 vs vPM vs Broca's area

Statistical analysis was run to compare the MEPs latency obtained by stimulating, with the HF-DES, M1 and vPM. Within each group of muscles (oro-facial and hand-arm), a Kolmogorov-Smirnov test showed that the latencies were normally distributed.

Single subject analysis. First, a Student's t test was used to assess statistical differences for different muscles subsample (Subsample A, oro-facial muscles: OO and MYLO; Subsample B, hand-arm muscles: EDC, ADM, APB, FDI) at single subject level. We include only muscles represented at list with five trials in both areas. We did not include responses from a muscle obtained only from one area in order to compare homogeneous subsamples for both M1 and vPM.

Population analysis. At population level two different analysis were performed.

A) In non-standardized analysis the comparison between areas were performed using latency expressed in milliseconds. A Student's t test was used when comparing paired sets of data, and the ANOVA test for analysis of data in >2 categories. For multivariate analysis of latency, a Generalized Linear Model (GLM) analysis of variance was used to test the factor brain area (vPM and M1), muscle (subsample A, and subsample B) and subject variability. We included in the model interaction terms between independent variables. We performed post-hoc tests using Bonferroni correction.

B) In standardized analysis, we applied same statistical procedure performed before but the latencies in millisecond were converted in Z score within each patient for each muscle independently by area, minimizing possible discrepancies due to variation in conduction path due to different body size. Moreover, a Student's t test was used to assess statistical differences for subsample A and B dependently by train of stimulation applied (one-pulse and multiple-pulse).

BOX 2. Pattern of activation of oro-facial muscles in *natural performance*

In order to compare the speech interferences induced by the LF-DES with the *natural performance* to infer the functional properties of Broca's area, vPM and M1 in speech production, it was necessary to disclose the pattern of oro-facial muscle activation in *natural performance*, i.e. which are the muscles significantly activated and which are not recruited in *natural performance*. This analysis was needed to determine whether the LF-DES induced a motor impairment on phono-articulatory muscles recruited in *natural performance*, or exerted an effect also on those not recruited to accomplish the task.

For each analysed muscle, the activated state was selected with respect to the resting state by comparing with a statistical analysis, the parameters monitoring the muscle activity (PS area and RMS mean) in the two conditions (BG-EMG *vs natural performance*). PS area and RMS mean were compared in the two conditions at level of single subject for both speech tasks separately, by using the Mann-Whitney U test (non-parametric test). Muscles failing to show a significant difference in the two conditions (*natural performance vs* BG-EMG) were considered to be inactive during phono-articulation.

Analysis of the muscle activation in denomination of different words

In an ideal experimental setting aimed at characterizing the specific alterations in muscular activity, occurring during the speech tasks during LF-DES stimulation, the EMG activation during LF-DES interference should be compared with the *natural performance* when the patient denominates the same word. However, the clinical setting did not allow for this procedure. In fact, in compliance with clinical needs, the patients denominated different words during the tasks. In such setting, it is mandatory to demonstrate that the pattern of motor unit recruitment of phono-articulatory muscles used by the patients to articulate different words was comparable: only when this condition is verified, the comparison of the *natural performance vs* performance during LF-DES is allowed irrespectively to the pronounced word (i.e. considering the different words as similar). To this aim the PSs curve of the EMG signals related to *natural performance* (in both tasks considered separately), for each recorded muscles, were compared two by two by calculating, for each comparison, the Correlation Coefficient (CC). The Correlation Coefficient, also called Pearson's Correlation index, is mathematically defined as the ratio between the covariance of variables and the product of their standard deviations or:

$$\rho_{X,Y} = \frac{\operatorname{cov}(X,Y)}{\sigma_X \sigma_Y}$$

where cov(X,Y) is the covariance between X and Y and is the standard deviation of X.

Due to possible biases due to interference external to the experiment, in order to use the CC, the areas of PSs corresponding to each word pronounced was normalized for each muscle. By normalizing the areas, the frequency distribution is expected to change mainly as a function of the motor strategy. Normalization was performed by calculating at first the maximal frequency value (maximal PS peak, PSmax). Then each point of the curve (composed by 500 points) was related to the peak of the curve, by calculating the ratio between the two values (PSvalue/PSmax). As a result, the frequencies are not expressed as absolute values but as percentage of the maximal frequency (values are indeed comprised between -1 and 1). Once the PSs curves were normalized, it was possible to compare the curves of different PSs. Each PS, in this study, was a curve made of 500 points, one for each frequency between 1 and 500 Hz. To compare two curves, each point of one curve was compared to the corresponding point on the other one. The point-to-point comparison was performed by calculating the log10 of the ratio between two curves:

log10 (a/b), where "a" and "be" represent corresponding points on the two spectrums.

The CC was hence calculated as:

[covariance of the [log10(an/bn)]] / [product of the standard deviation of the [log10(an/bn)]]

The CC has a value in between -1 and +1. The correlation was considered negative if the value was <0; absent if equal to 0; weak for values between 0 and 0,3; moderate for values between 0,3 and 0,7 and strong if the CC is >0.7. For this reason, the cut-off value to consider two different words as comparable was set at values >0.7.

As a result of this analysis emerged that for all the comparison performed (the PSs curve of the EMG signals related to *natural performance* -in both tasks considered separately-), for each recorded muscles, CC was >0.7. We could thus safely compare, for each muscle, the EMG *natural performance* with that articulated during the application of the LF-DES on cortical motor areas. Multiple repetitions of the same word during application of stimulation to different sites are indeed in contrast with the clinical requirements, which are prevailing in the intraoperatory setting.

DES-induced interference effects during task performance

Single subject analysis. Statistical analysis was run to compare the effect of Broca's area, vPM and M1 stimulation on the EMG activation during single words production (counting and object picture naming tasks), in OO, MENT and MYLO muscles (contra- and ipsilateral to stimulate hemisphere) with their EMG activation during *natural performance.*

Comparison was computed for each word denominated in the two tasks (counting and object picture naming) and in the two conditions (*LF-DES interference* and *natural performance*), for five EMG parameters of each recorded muscles (mean and peak values for RMS; mean and median frequencies and area under the curve for PS). Statistical analysis was computed with Statistica 7.1 software. Each parameters were compared by means of the Mann-Whitney U test, at level of single subject. The significance level adopted was of 5% ($p<0.05$).

Population analysis. Based on the results obtained on single subject analysis it was possible to apply a population analysis on data obtained by stimulating vPM (see Results section) and M1. Population analysis was performed with the following aims:

A) To evaluate whether the pattern of interferences induced by the LF-DES onto vPM during object picture naming and counting tasks were comparable. This analysis was not applied onto M1, because only 1 patient stimulated on M1 performed both language tasks.

We considered the patients that performed both tasks and in which the LF-DES was applied onto the same stimulation site. In these patients, we have previously calculated the five EMG parameters (see above) related to *LF-DES interference*, considering separately the two speech tasks. Then, we used a Generalized Linear Model (GLM) to compare each EMG parameters related to *LF-DES interference* in two speech tasks (counting and object picture naming), independently by other factors (univariate

analysis), and considering (multivariate analysis) separately the analysed-muscle (OO, MENT, MYLO) and hemi body of recorded muscle (contra- and ipsilateral).

B) To compare the effect of DES on ipsi and contralateral muscles' activity. Since we recorded the phono-articulatory activity of contra- and ipsilateral muscles, by using the same method applied in analysis A, we compared the effect of LF-DES applied on vPM and M1, separately, on contra- and ipsilateral muscles. A GLM was used to test the LF-DES effect on vPM and M1 independently by other factors (univariate analysis), and considering (multivariate analysis) as a factor the muscle (OO, MENT, MYLO).

3D Map

Identification of oro-facial and hand-arm representations in M1, vPM and Broca's area

In 21 patients, we analysed the distribution of motor responses obtained by HF-DES stimulation onto the so-called "positive sites", i.e. the eloquent cortical sites responding to stimulation with a MEP. Stimulation of cortical sites eliciting responses in only one muscle of those recorded (either an oro-facial or a hand-arm muscle) were denominated *Single Positive sites*; the cortical sites from which responses in multiple muscles were obtained were defined as *Multiple Positive Sites*. Stimulation of a Single Positive site therefore resulted in oro-facial or hand-arm responses, i.e. activation of a single muscle in only one of the two effectors tested. Stimulation of the Multiple Positive Sites resulted in Simple Muscle combination responses when activating several muscles within the same effector (oro-facial or hand-arm) and in Complex Muscle combinations when activating several muscles involving both the effectors simultaneously (oro-hand responses). We considered responses evoked with the minimal stimulation parameters combination, i.e. minimal intensity and minimum number of stimuli.

Map of Positive Sites

For each patient, the reconstruction of the exact position of the Positive Sites onto the cortex was computed. During intraoperative mapping the exposed craniotomy was entirely video recorded and the MRI coordinated of the Positive Sites were acquired by means of the neuronavigator system. To report the exact position of the Positive Sites on the 3D MRI cortical surface of each patient the following procedure was adopted. The post-contrast T1-weighted sequence of each patient (the same loaded onto the neuronavigator during surgery) was used to perform the cortical surface extraction and surface volume registration computed with the Brainsuite 15b dedicated software (Shattuck and

Leahy 2002) and then the results were loaded into Brainstorm (MatLab Tool Box; Tadel et al. 2011), which is documented and freely available for download online under the GNU general public license (http://neuroimage.usc.edu/brainstorm).

With the aid of Brainstorm, the exact position of the Positive Sites coordinates was labelled onto the 3D patient's MRI. Subsequently the 3D MRI and the labelled Positive Sites were co-registered to the MNI space system (ICBM 152) with the aid of Brainstorm. This procedure allows reporting each Positive Sites to the MNI coordinates space system. Coordinates of each Positive Site were then labelled onto the ICBM 152 to create a 3D reconstruction of the stimulated hemisphere where the Positive Sites are reported in a colour code. Each Positive Site was associated with a specific colour based on the response muscle evoked, to obtain a final picture of the somatotopic representation of the effectors both in vPM and in M1. This analysis was performed in 19 out of 21 patients (total number of Positive Sites in M1 = 40 and in vPM = 46) where both the video and the navigation system recordings were available during cortical stimulation. A probabilistic map in the MNI coordinate system was then obtained using MNI values of each Positive Site. Graphical reconstruction was performed with a dedicated script based on probability density function implemented in MatLab (MathWorks 2009b).

Comparison of DES-induced interference effects in M1, vPM and Broca's area

For each patient, the reconstruction of the exact position of the stimulation sites onto Broca's area, vPM and M1 was computed, including negative sites (points where LF-DES did not induce an interference with speech production). During intraoperative mapping, the coordinates of these sites were acquired by means of the neuronavigator system. To report the exact position of the sites on the 3D MRI surface of the patients, we adopted the following procedure: the MRI-T1 volume of each patient was used to perform the cortical surface extraction and surface volume registration computed with the dedicated software FreeSurfer, the results were then loaded into Brainstorm. With the aid of Brainstorm, the exact position of the points coordinates was labelled onto the 3D patient's MRI and subsequently the 3D MRI and the labelled points were co-registered to the MNI space system (non-linear ICBM 152). This procedure allowed to report each points to the MNI coordinates space system. Coordinates of each points were then labelled onto the ICBM 152 to create a 3D reconstruction of the stimulated hemisphere. A probabilistic map in the MNI coordinate system was then obtained using MNI values of stimulation sites. Graphical reconstruction was performed with a dedicated script based on probability density function implemented in MatLab (MathWorks 2009b).

HARDI reconstruction of the fibers extremity reaching Broca's area

For each patient in which Broca's area was stimulated during speech tasks (20 out of 70), the FA maps were co-registered to the anatomical images, obtained in pre-operative phase (see above), and to the FSL 2×2×2 mm3 resolution Montreal Neurological Institute (MNI) atlas using FSL linear and non-linear transformations (FMRIB's FLIRT and FNIRT registration tools). Density maps of the end points of each fibre tract (arcuate *fasciculus* [AF], superior longitudinal *fasciculus* component II [SLF-II], SLF component III [SLF-III], SLF component temporal-parietal [SLF-tp], inferior frontal-occipital *fasciculus* [IFOF], uncinate *fasciculus* [UF] and frontal aslant tract [FAT], see above) were saved in the patients' native space using TrackVis and thresholded to obtain binary masks containing all voxels that were visited by at least one streamline in the q-ball residual-bootstrap tractography. These masks were spatially normalized to the MNI space using the linear and non-linear transformations derived from the co-registration of the FA maps to the FSL MNI atlas. All patients' end point maps in the MNI space for each tract were summed to visualize the distribution of the tract terminations and their overlap with intraoperative stimulation sites normalized to the MNI space. A 3D rendering of the end points of the different tracts was obtained using the FSL 3D viewer. This analysis was computed by a neuroradiologist, Dr Antonella Castellano, collaborating to this project.

3. *Results*

The aim of PhD project was to investigate the functional properties of the cortical areas involved in sensory-motor control of speech by acting on the phono-articulatory apparatus. The three areas, historically associated to motor control of speech, were Broca's area, ventral pre-motor (vPM) and primary motor (M1) cortices.

The study was conducted in 70 patients affected by gliomas affecting the dominant language hemisphere (the left hemisphere in all our patients) and proximal, but not infiltrating, the areas of interest. A direct investigation of Broca's area, vPM and M1 in humans was performed in this project by the unique setting of tumour removal with the aid of the *brain mapping* technique allowing, for clinical purposes, the stimulation of the three areas, while recording the electrical activity (EMG) of some phono-articulatory muscles involved in speech production (see Materials and Methods section). As described in details in the Materials and Methods section, the study was designed in sub-sequent phases:

a) First: the identification of the precise site on vPM and M1 hosting the oro-facial representation (expected to control the phono-articulatory apparatus), by distinguishing it from the hand-arm representation. To this aim, EMG responses to LF- and HF-DES stimulation were recorded. Despite according to a previous study of our group, no motor responses can be elicited by Broca's area stimulation, the same approach was used to explore Broca's area.

b) Second: the assessment of the effect of LF-DES delivered on vPM, M1 and Broca's area during task speech (counting and object picture naming) performance. During the stimulation, the electrical activity of muscles involved in phono-articulation and the clinical output were monitored. The offline analysis of the muscle activity allowed to characterize and compare the pattern of motor unit voluntarily recruitment during the performance of speech tasks in absence of stimulation (*natural performance*), with those occurring during tasks performance when the stimulation was applied on three cortical areas (*LF-DES interference*). By analysing the specific alterations in the pattern of motor unit recruitment, associated to the stimulation of the three different cortical areas, some speculation on the role exerted by M1, vPM and Broca's area in motor control of speech production were discussed.

3.1 IDENTIFICATION OF ORO-FACIAL AND HAND-ARM REPRESENTATIONS IN M1, vPM AND BROCA'S AREA

To localize the oro-facial and hand-arm representations of Broca's area, vPM and M1, first we applied onto them the Direct Electrical Stimulation (DES), recording simultaneously the muscle activity of some oro-facial and hand-arm muscles and the position of stimulation sites. DES was applied when oro-facial and hand-arm muscles were at rest and then in a pre-activated state. The DES paradigms used were two: LF (Low Frequency) and HF (High Frequency). With either paradigm, a qualitative motor response could be observed upon DES application onto vPM and M1, but not in Broca's area. However, we opted to apply the quantitative analysis only to data obtained during stimulation carried out following the HF-DES paradigm, in order to define with more precision and sophisticated technique the functional border between oro-facial and hand-arm districts in the three areas.

The qualitative results have been published in Cerri and colleagues (2015), while the quantitative analysis in Fornia and colleagues (2016).

Comparison of motor responses in M1, vPM and Broca's area

Motor output of M1, vPM and Broca's area with LF-DES in resting and pre-contracted conditions

The LF-DES was applied onto Broca's area, vPM and M1 at rest and in pre-contracted state in 5 patients (out of 70).

In these subjects, the LF-DES on M1 elicited contralateral muscle contractions in both conditions, resting and pre-contracted, for both effectors (hand-arm and oro-facial muscle) in distinct sites. EMG responses in contralateral resting and pre-contracted hand-arm and oro-facial muscles in sites consistent with those documented as active during movement of the same bodily districts by the pre-operative fMRI. In resting state, a progressively increasing of muscle recruitment emerged from the background activity (**Fig. 3.1 A**, left panel). When stimulation was applied in the pre-contracted condition, a faster recruitment in contralateral muscles was observed (**Fig. 3.1 B**, left panel), as expected by the summation of the voluntary with the LF-DES-induced corticospinal drive.

The LF-DES applied onto vPM elicited motor responses in resting hand-arm muscles only in one subject (**Fig. 3.1 A**, middle panel), with a very slow EMG onset, suggesting that vPM is a less excitable area with respect to M1, probably requiring multisynaptic summation to trigger a response. The stimulation of vPM in tonically pre-activated muscles resulted in a disruption of the ongoing

EMG activity, usually with a concomitant recruitment of other muscles not involved in the tonic contraction, rather than in a powerful muscle recruitment (**Fig. 3.1 B**, middle panel). This effect was observed only when the stimulation involved oro-facial muscles.

When the LF-DES was applied onto Broca's area, it failed to induce any motor effects both in resting (**Fig. 3.1 A**, right panel) and in pre-activated state in all subjects (**Fig. 3.1 B**, right panel), questioning its role in eliciting a motor output.

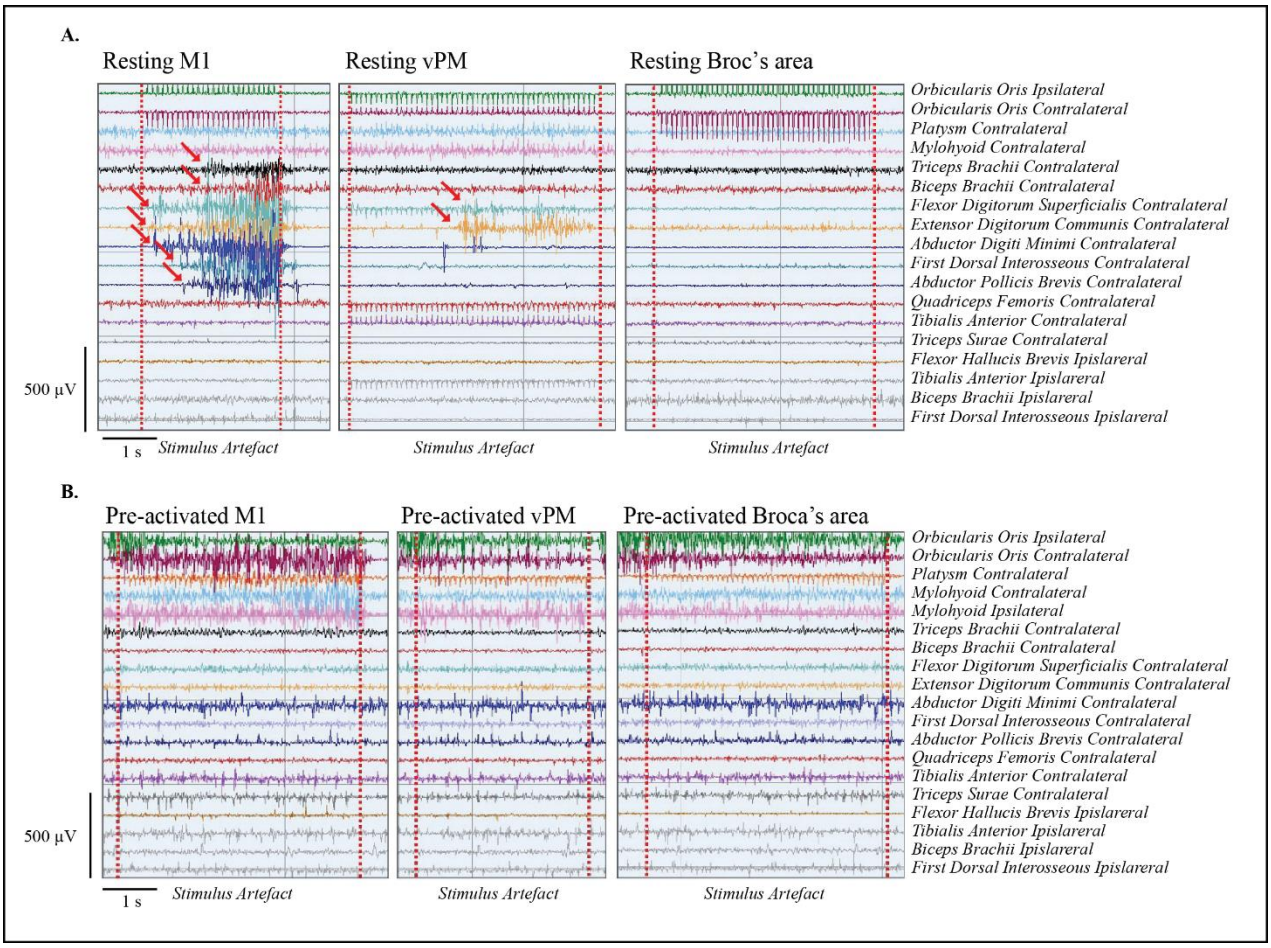

Figure 3.1 The LF-DES applied on M1 (left), vPM (middle) and Broca's area (right) in rest (**A**) and pre-activated (**B**) conditions. The red arrows indicate the EMG onset of recruit muscles. Stimulus onset and duration are indicated with dotted lines. The recorded muscles are indicated at right of panels.

From *Cerri et al., 2015 modified*.

Motor output of M1, vPM and Broca's area with HF-DES in resting and pre-contracted conditions

Trains of the HF-DES were applied at rest in 27 patients (out of 70) and in pre-contracted state in 10 patients (out of 70). In these subjects, the HF-DES on M1 elicited motor evoked potentials (MEPs)

both in resting and in active target muscles, according to the somatotopic cortical representation. The number of stimuli needed to elicit MEPs varied from 1 in pre-activated muscles, to 1-2 (rarely 3) in resting muscles (**Fig. 3.2 A**).

The HF-DES on vPM required a higher number of shocks (3 to 5, rarely 1-2) to elicit responses in both hand-arm and oro-facial muscles (**Fig. 3.2 B**), suggesting again that vPM is a less excitable area with respect to M1.

The HF-DES of Broca's area (**Fig. 3.2 C**) always failed to elicit any response in any muscle, both in resting and in active state, even when increasing the number of shocks (up to 7), suggesting again that Broca's area does not show a motor output.

Conclusively, from a qualitative standpoint, the LF-DES and the HF-DES induced the same effects onto three areas: motor responses are elicited by stimulating M1 and vPM while no motor responses can be elicited by stimulating Broca's area.

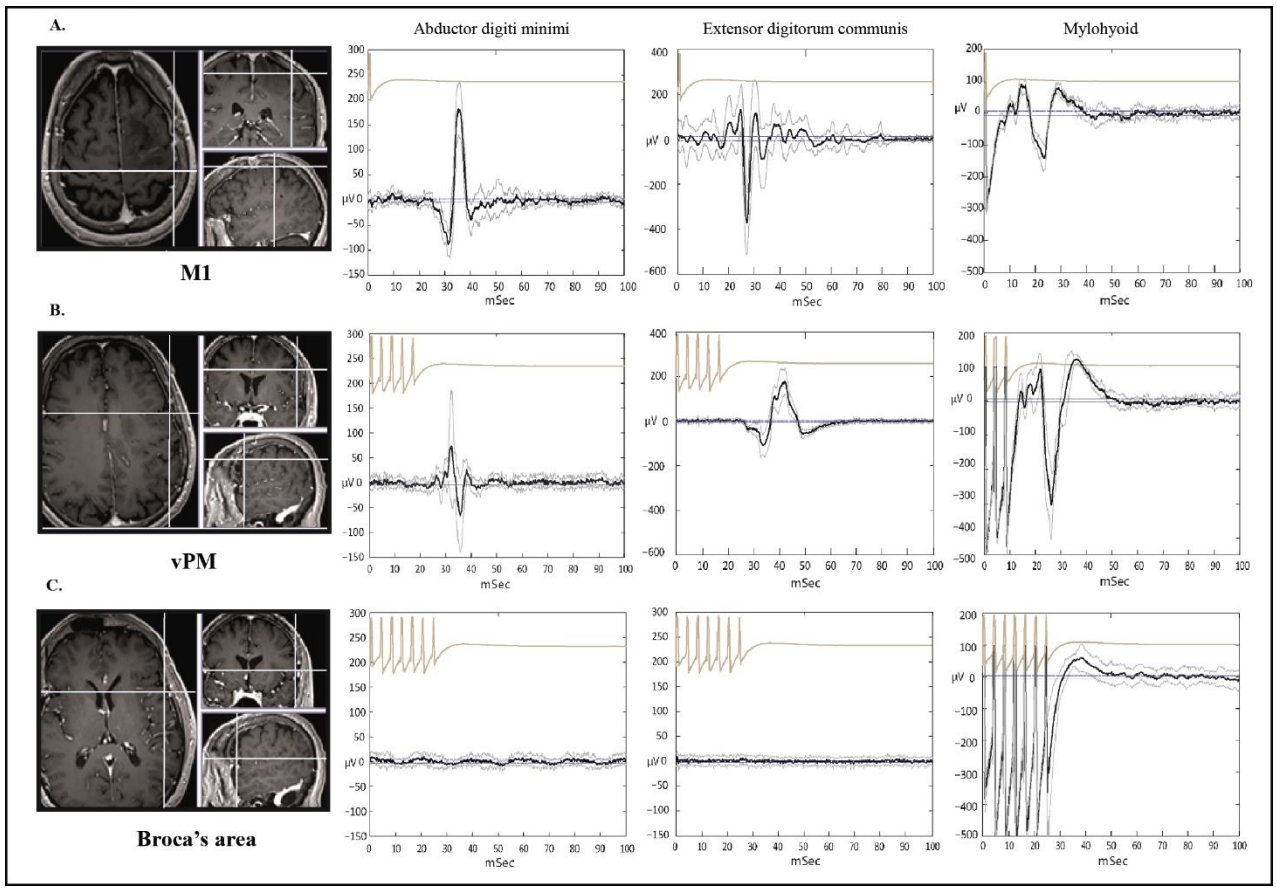

Figure 3.2 The HF-DES applied on M1 (**A**), vPM (**B**) and Broca's area (**C**) in rest condition in one exemplificative subject (pre-contracted state not shown). On the right, MEPs recorded in *abductor digiti minimi*, *extensor digitorum communis* and *mylohyoid* muscles following the stimulation of M1 (1 shock; average of 10 trials), vPM (5 shocks, inter stimulus interval: 4 ms in *abductor digiti minimi*, *extensor digitorum communis* ; 3 shocks in *mylohyoid* , inter stimulus interval: 4 ms; average of 15 trials) and Broca's area (7 shocks, inter stimulus interval 4 ms; average of 20 trials) are shown. On the left, the sites of stimulation eliciting MEPs in the hand region of M1, hand-arm/oro-facial region of vPM and unaffected Broca's region are shown.

From *Cerri et al., 2015 modified*.

Analysis of MEPs: M1 *vs* vPM (*vs* Broca's area)

MEPs were obtained stimulating vPM and M1, while no responses were obtained by stimulating Broca's area, therefore the following analysis was only applied onto vPM and M1.

We recorded MEPs from vPM and M1 in 21 patients out of 27 considered for this analysis, depending on the accessibility of areas in the surgical flap. In these patients, the number total of stimulation sites on stimulated hemisphere was 49 in M1 and 48 in vPM (total 97 sites), while the total number of recorded MEPs were 1931 from M1 and 1664 from vPM (total 3595 MEPs).

Stimulation sites and somatotopic organization of muscle responses

- In M1: 26/49 identified sites (53%) were single positive sites, eliciting responses in a single muscle (N = 8 in the oro-facial group and N = 18 in the hand-arm group), 21/49 identified sites (43%) were multiple positive sites, eliciting simple muscle combination responses within the same effector (N = 5 in the oro-facial group, and N = 16 in the hand-arm group: N = 9 in 2 muscles and N = 7 in all hand-arm recorded muscles), while 2/49 identified sites (4%) were again multiple positive sites, but eliciting complex muscle combination responses activating muscles belonging to different effector groups, so called "oro-hand responses" (**Fig. 3.3**).
- In vPM: 18/48 identified sites (38%) were single positive sites (N = 9 in the oro-facial group and N = 9 in the hand-arm group), 13/48 identified sites (27%) were multiple positive sites, eliciting simple muscle combination (N = 6 in the oro-facial group, N = 7 in the hand-arm group: N = 4 in 2 muscles and N = 3 in whole hand-arm recorded muscle), while 17/48 identified sites (35%) were again multiple positive sites, but eliciting complex muscle combination (**Fig. 3.3**).

By comparing the relative percentage of the three different categories of responses (single muscle responses, simple combinations and complex combinations of muscle responses) recorded in the two regions, M1 showed a clear prevalence of single muscle responses and simple combination muscles responses (53% and 43%, respectively), while vPM showed all categories of response in similar proportions (38%, 27% and 35%, respectively). Complex muscle combinations were almost completely absent in M1 (4%).

The stimulation sites related to single muscle responses, simple combinations and complex combinations of muscle responses were reported on a 3D reconstruction of the brain left hemisphere based on the MNI template (ICBM 152, see Materials and Methods section). This reconstruction was necessary to show: i) the distribution over the M1 and vPM cortices of the positive sites, and ii) the somatotopic organization of muscle responses on the two areas (**Fig. 3.4 A**).

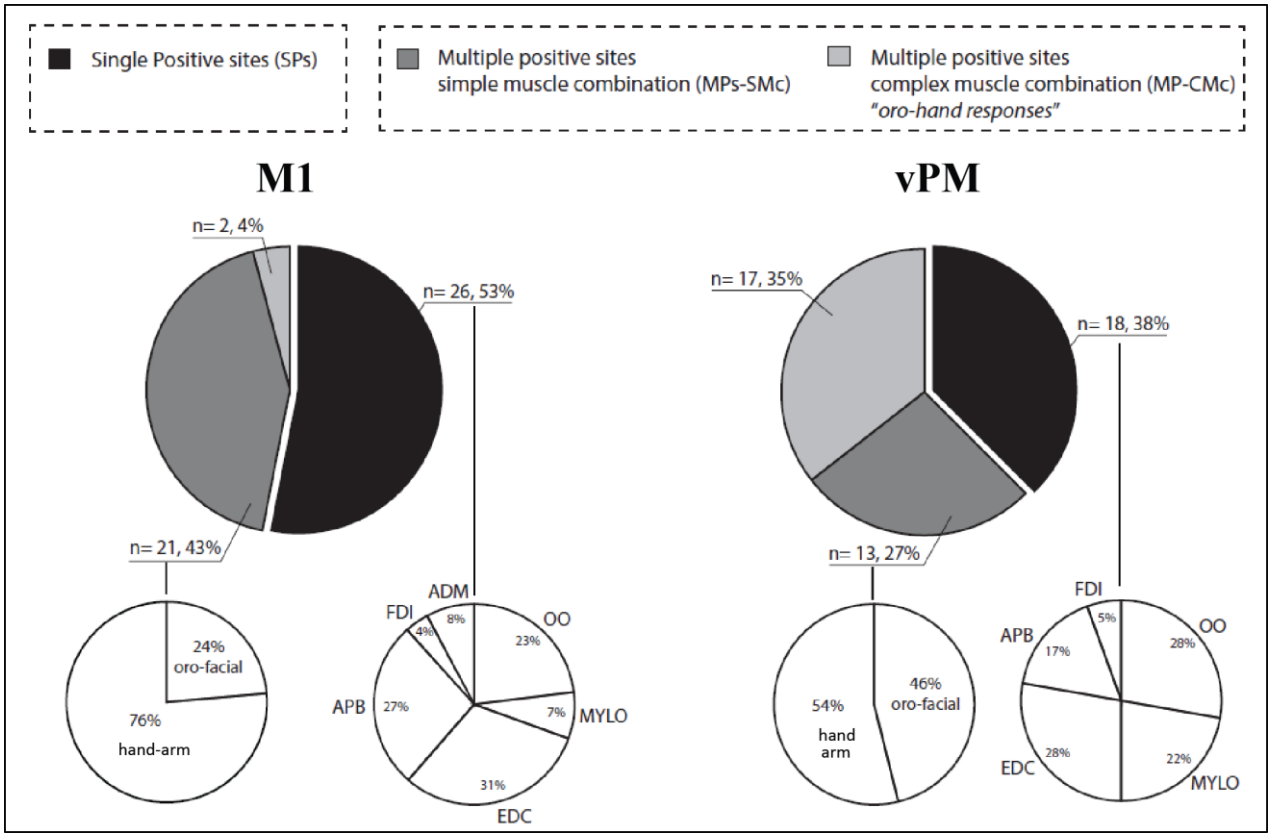

Figure 3.3 Frequency and type of positive sites in M1 and vPM. MEPs from Broca's area were not obtained. The graphs report the distribution of the motor responses obtained by HF-DES from so-called *positive sites*. The cortical sites eliciting responses in a single muscle (either an oro-facial or hand-arm muscle) are denominated Single Positive sites (SPs); the cortical sites eliciting responses in multiple muscles are defined as Multiple Positive sites (MPs). The stimulation of the MPs resulted in Simple Muscle combination responses (MPs-SMc) when activating several muscles within the same effector (oro-facial or hand-arm) and Complex Muscle combinations (MPs-CMc) when activating several muscles involving both the effectors ('oro-hand responses').
Oro-facial muscles: OO = *Orbicularis Oris*, MYLO = *Mylohyoid*; Hand-arm muscles: EDC = *Extensor Digitorum Communis*, APB = *Abductor Pollicis Brevis*, FDI = *First Dorsal Interosseous*, ADM = *Abductor Digiti Minimi*.

From *Fornia et al., 2016 modified*.

In M1, two distinct regions were identified: one, more dorsal, hosting the representation of the hand-arm region (black dots) and the other, more ventral, with an oro-facial representation (light green dots). In vPM, three different somatotopic representations were identified: the most ventral sector was not responsive to stimulation, while more dorsal-ventral to this area were observed almost exclusively oro-facial responses (dark green dots). By stimulating more dorsally, up to the inferior frontal *gyrus*, pure hand-arm responses (blue dots) were prevalent, while, between these two sectors, a "transition zone" emerged, characterized by the presence of multiple positive sites eliciting complex muscle combinations, so-called "oro-hand" responses. The stimulation of this last sector elicited motor responses with similar amplitude in different muscles of the hand-arm and oro-facial groups

simultaneously. These multisegmental responses were very rarely observed upon stimulation of M1 and, in the few sites giving rise to such responses, there was a clear difference in amplitude, with hand-arm motor responses always being greater than oro-facial, rather suggesting that stimulation was delivered in the M1 hand area, right at the border with the M1 oro-facial area. In this case, indeed, the stimulus elicited clear hand responses and, possibly due to the spread of current to the neighbouring oro-facial groups, evoked small oro-facial responses, suggesting that M1 does not host an area with an actual co-representation of both effectors.

The **Figure 3.4 B** shows the somatotopical probabilistic map (lateral view of the stimulated hemisphere) obtained by means of MNI coordinates of each positive site. The density estimation for each site is reported with a colour code indicating the probability level of site density obtained by interpolating MNI Y and Z values. Due to the tumour location and cortical surface made available by the flap during cortical mapping, we could not systematically investigate all regions (vPM and M1)

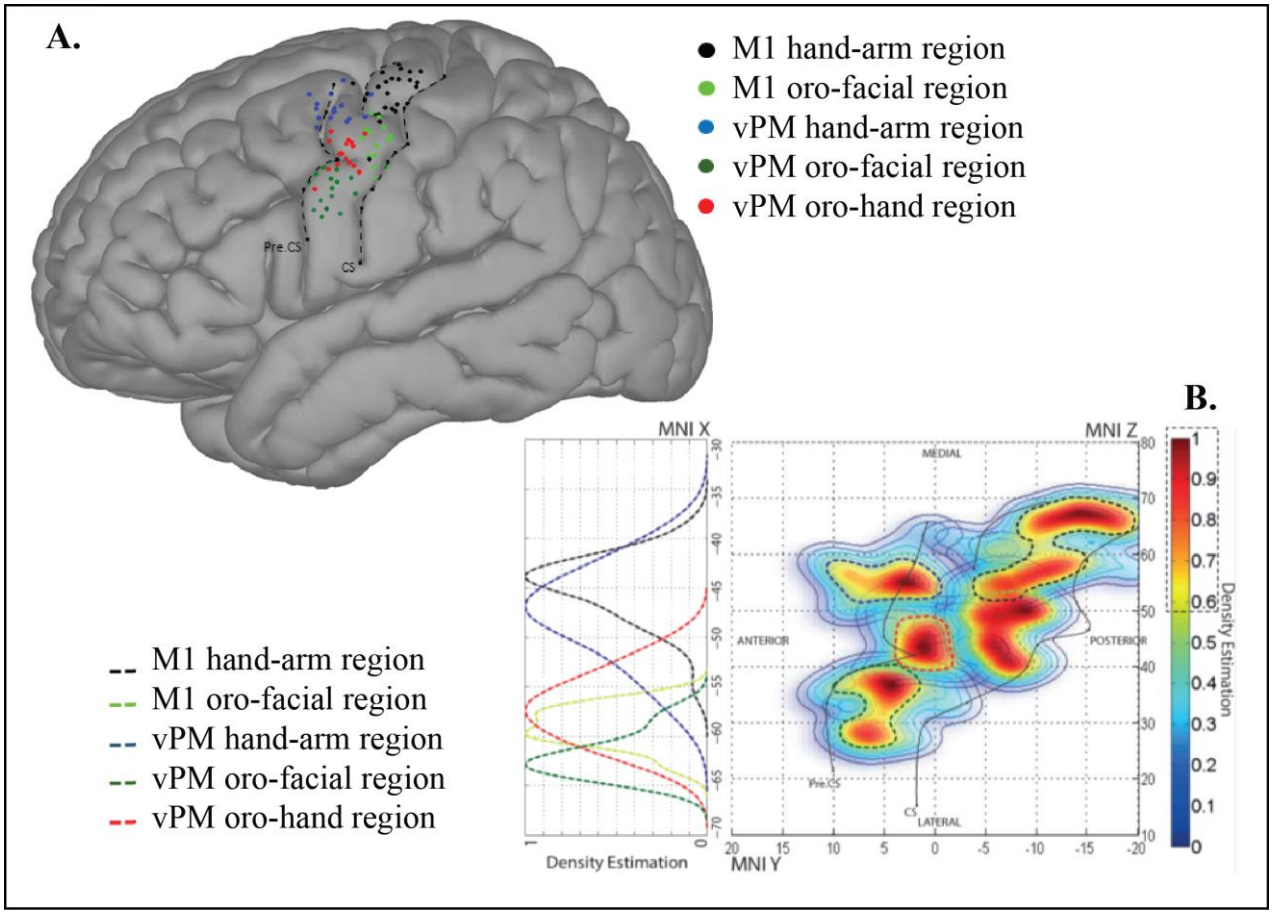

Figure 3.4 Cortical distribution of MEPs obtained from vPM and M1. **A)** MNI template (non-linear ICBM152) of left hemisphere: each dot represents a single positive site and the colour represents its somatotopic group, indicated at right**. B)** Two-dimensional probabilistic map based on the MNI coordinates (Y and Z) for each site present in the MNI template and on the left side distribution for each somatotopic group of the X values in the site with the higher probability density value. Colour bar represents the density estimation value. Colored dashed lines correspond to a specific somatotopic muscle group, indicated at left.
CS: Central Sulcus; PreCS: Pre-Central Sulcus.

From *Fornia et al., 2016*.

in all patients (N = 21). The localization of the sites on M1 and on vPM had to be confirmed by the analysis of the latency of the responses (see next paragraph), which was critical in assigning with precision the motor response as originating from M1 rather than from vPM.

Latency of MEPs evoked by stimulating vPM and M1

In studies on non-human primates it is reported that the electrical stimulation of M1 can activate the corticospinal cells either directly, evoking earliest direct-waves (Patton and Amassian, 1954), or by trans-synaptic activation via cortical interneurons, evoking indirect-waves (Amassian et al., 1987). Conversely, there is no evidence that stimulating vPM gives rise to a direct-wave in the corticospinal tract (Shimazu et al., 2004; Maier et al., 2013). This suggests that the connections of vPM with the spinal cord must be indirect and involve, for example, specific cortico-cortical connections with M1, which in turn sends numerous direct projections to spinal motoneurons (Morecraft et al., 2013). If in humans the connection between vPM and the spinal cord is indirect as well, then MEPs evoked by stimulating vPM should have an increased latency compared to those evoked stimulating M1.

In our patients, a total of 3595 MEPs were recorded, including 1931 trials from M1 stimulation and 1664 from vPM. These MEPs were recorded in 21 subjects (33.7 ± 17.9 trials for muscle for each subject) in six muscles (number of trials ± standard deviation -SD-: 300 ± 135.7 trials per muscle). We divided MEPs in two subsample, based on muscle district to which belonged MEPs: Subsample A for MEPs evoked in oro-facial muscles (OO and MYLO), Subsample B for MEPs evoked in hand-arm muscles (*abductor digiti minimi*, ADM, *extensor digitorum communis*, EDC, *abductor pollicis brevis*, APB, and *first dorsal interosseous*, FDI).

- Subsample A: 1143 trials were recorded in oro-facial muscles, 296 from M1 and 847 from vPM. The OO was recorded in 15 patients (196 trials from M1 and 388 from vPM, respectively) and the MYLO recorded in 9 patients (100 trials from M1 and 459 from vPM, respectively).
- Subsample B: 2452 trials were recorded in hand-arm muscles, 1635 from M1 and 817 from vPM. The EDC was recorded in 8 patients (482 trials from M1 and 300 from vPM, respectively), the FDI in 8 patients (332 trials from M1 and 258 from vPM, respectively), the APB in 7 patients (410 trials from M1 and 139 from vPM, respectively) and the ADM in 8 patients (411 trials from M1 and 120 from vPM, respectively).

We compared MEPs latency obtained by applying HF-DES to M1 and vPM and found differences across muscles and conditions (single *vs* multiple pulse). The mean latency values between two muscle districts ranged from 1.2 to 2.4 ms in the subsample A (oro-facial muscles), and from 2.0 to 2.7 ms in the subsample B (hand-arm muscles) (**Fig. 3.5 B**). Student's t test and U-test showed similar levels of significance ($p<0.05$). Regarding the different stimulation conditions: i) in the single pulse

analysis, all patients but two in the subsample A, and two in subsample B showed a statistical difference ($p<0.05$) (**Fig. 3.5 A** at left) between the latencies of MEPs evoked from the two areas; ii) in the multiple pulse analysis, all patients but one in subsample A and one in the subsample B showed a statistical differences ($p<0.05$) , at difference of other patients (**Fig. 3.5 A** at right).

Concerning analysis at population level, a non-standardized latency univariate analyses demonstrated that responses in hand-arm muscles from M1 had significantly shorter latencies than responses from vPM (M1 = 22.4 ± 3.3 ms *vs* vPM = 23.5 ± 3.0 ms; $t(1743.8)=8.3$; $p<0.001$). The same analysis confirmed this result for the oro-facial group (M1 = 10.7 ± 1.7 ms *vs* vPM = 12.9 ± 1.9 ms; $t(1141)=18.1$; $p<0.0001$). In this group, the latency shift was different across individuals [$F(14,1128)=91.4$, $p<0.001$], but not between the two muscles (OO = 12.3 ± 2.44 ms *vs* MYLO = 12.4 ± 1.7 ms; $t(1032.4)=-0.5$; $p=0.6$).

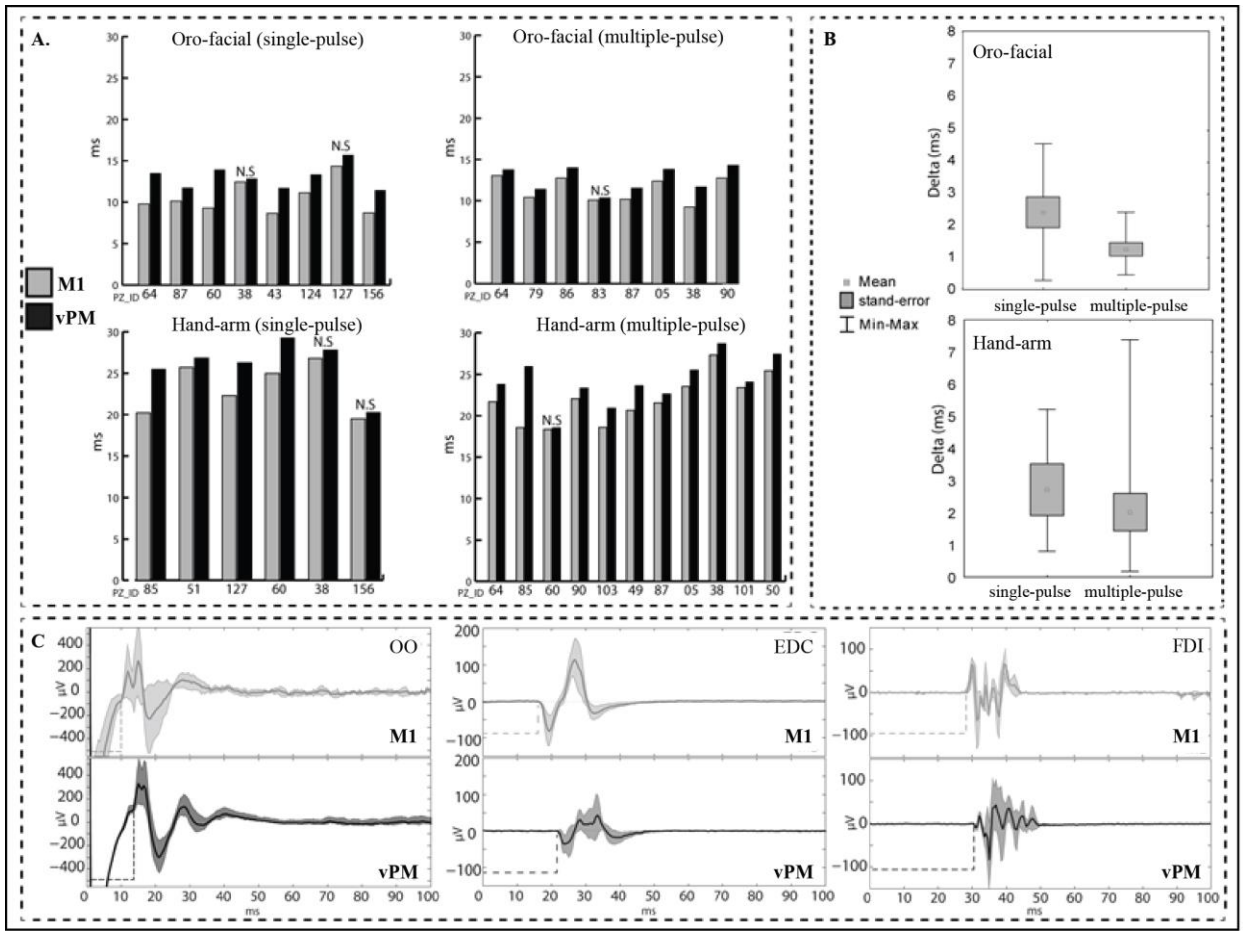

Figure 3.5 A) Latency analysis of MEPs obtained with single and multiple pulses in oro-facial and hand-arm muscles at the single subject level. **B)** Mean latency differences ('Delta ms') of MEPs evoked from vPM and M1 with single and multiple pulses calculated for each patient and for each group of muscles. Average value, standard error and min/max of Delta (in ms) are shown. **C)** Examples of averaged MEPs with standard deviations (10 trials, single pulse) from three muscles evoked by stimulation of M1 (above) and of vPM (below). Dashed lines indicate the latency between the stimulus and the rising phase of the MEP.
NS = indicates that the difference between the latency of responses to M1 and vPM stimulation was Not Significant.

From *Fornia et al., 2016 modified*.

In the multivariate models, after adjustment for muscle type and subject, brain area was confirmed to be a significant predictor of the model in the oro-facial [F(1,1143)=376.8, p<0.001] and hand-arm group [(1,2452)=1086.2, p<0.001], with a good overall explanation of the two models (global R^2=0.8 and R^2=0.8, respectively).

Moreover, the analyses with standardized latencies were performed in order to get a more realistic picture of the trends of our data without the possible contamination caused by the presence among patients of variable and different body sizes (Livingston et al., 2010) such as to preclude a genuine global vision. Univariate analysis demonstrated that responses in hand-arm muscles from M1 had significantly differences respect vPM (M1= -0.329 ± 0.777 Z-score *vs* vPM = 0.73 ± 0.973 Z-score; t (2450)=-29.20; p<0.001). The same analysis confirmed this result for the oro-facial group (M1= -0.703 ± 0.965 Z-score *vs* vPM = 0.245 ± 0.874 Z-score; t(1141)=-15.65; p<0.001).

In the multivariate models, after adjustment for muscle type and subject, brain area was confirmed to be a significant predictor of the model in the oro-facial [F(1,1143)=376.8, p<0.001] and hand-arm subsamples [F(1,2450)=853.06, p<0.001](**Fig. 3.6 A**).

These results were confirmed also with univariate analysis performed for each muscle between areas (MYLO = M1 -0.622 *vs* vPM 0.135 t(557) = - 7.228, p<0.001; OO = M1 -0.745 *vs* vPM 0.376, t(582) = - 15.33, p<0.001; EDC = M1 -0.375 *vs* vPM 0.767, t(780) = - 19.347, p<0.001; APB = M1 -0.238 *vs* vPM 0.703, t(547) = - 10.589, p<0.001; FDI = M1 -0.503 *vs* vPM 0.47, t(588) = - 17.029, p<0.001; ADM = M1 -0.247 *vs* vPM 0.848, t(529) = - 11.977, p<0.001) (**Fig. 3.6 B**).

In the applied models, we included the latencies of responses obtained with a single pulse and the latencies obtained with trains of stimulation. Given that it is not possible to exclude that the neural elements recruited with a single pulse could be different from those recruited with a train of pulses, we performed the same statistical analysis aimed at evaluating the latency shift (Z scores) between M1 and vPM based on the stimulation paradigm, i.e. separating data obtained with a single pulse and those obtained with a train of pulses. This analysis confirmed, for both single and multiple pulses, a statistical difference between M1 and vPM in both muscle groups (latencies in response to a single pulse: subsample A, M1 -0.416 *vs* vPM 0.479, t(396)= -10.075 p<0.001; subsample B, M1 -0.172 *vs* vPM 0.448, t(512)= -6.621 p<0.001; latencies for multiple-pulse: subsample A, M1 -0.518 *vs* vPM0.138, t(734)= -7.580 p<0.001; subsample B, M1 -0.484 *vs* vPM 0.701, t(1404)= -27.073 p<0.001 (**Fig. 3.6 C**)).

For single pulses, the latency difference for M1 *vs* vPM evoked MEPs in oro-facial muscles ranged from 0.3 to 4.59 ms and in hand-arm muscles from 0.8 to 5.22 ms. For a train of pulses the latency differences were 0.47 to 2.44 ms for oro-facial and 0.19 to 7.38 ms for hand-arm muscles (**Fig. 3.5 B**).

From this analysis, it clearly emerges that the motor output of the human vPM is characterized by responses with a longer latency with respect to those originating from M1. The longer latency of the MEPs evoked by vPM stimulation excludes the possibility that responses obtained by the stimulation of this area result from the spread of current from vPM to M1.

The analyses on latencies of MEPs strongly supports the oro-facial and hand-arm representations of vPM and M1 illustrated by the 3D reconstruction reported above. Interestingly the most ventral portion of vPM seems not responsive to HF-DES, while more dorsally, three regions are recognized based on their somatotopic representation: from ventral to dorsal, a hand-arm, an oro-hand and an oro-facial from more mesial portion to lateral portion (**Fig. 3.4**). M1 (excluding the foot-leg

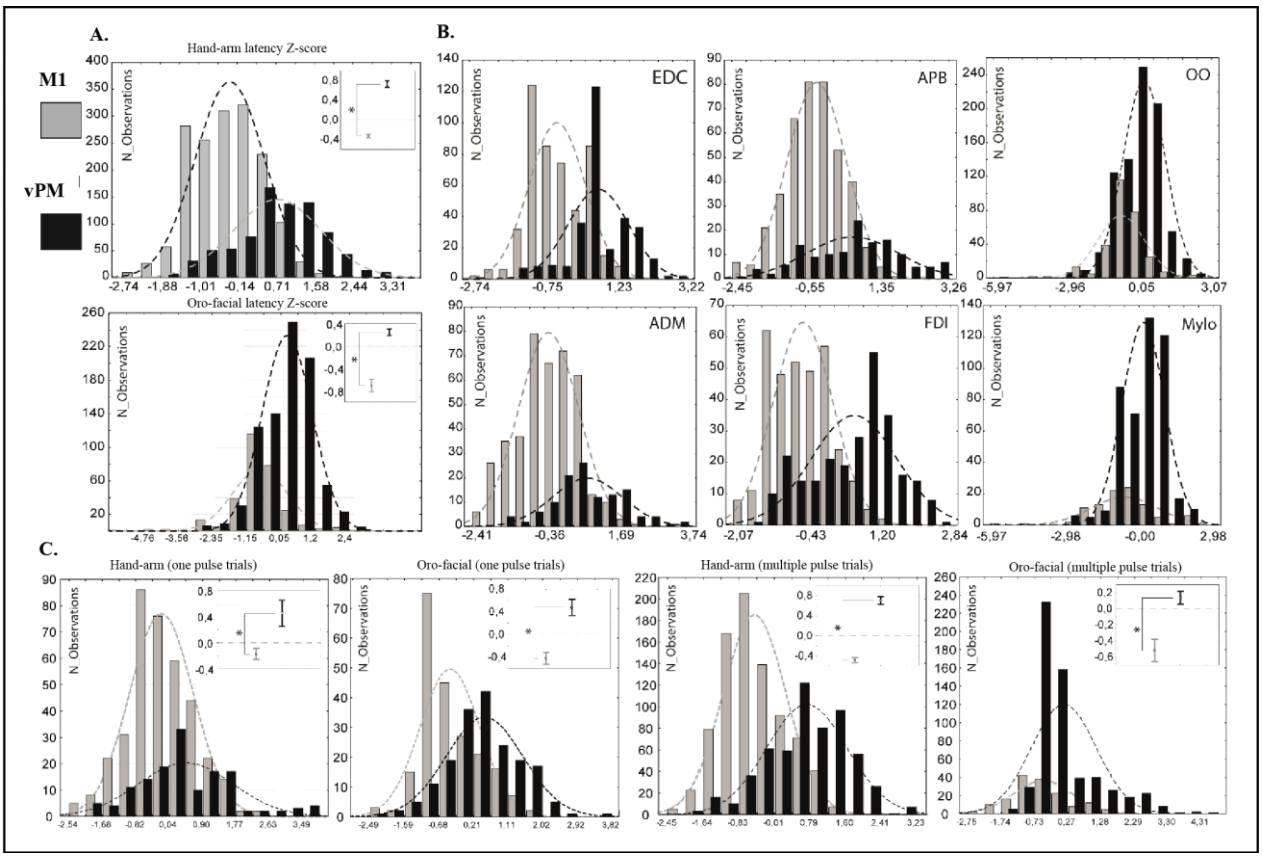

Figure 3.6 **A)** Distribution of the latencies of MEPs evoked from the two areas in subsample B (hand-arm) and subsample A (oro-facial). In order to show the distribution at population level, the latencies in milliseconds were standardized in Z score within each patient for each muscle independently by area. The dashed line coincides with the normal distribution of the MEPs latencies expressed in Z score. In each figure, the right-upper panel shows a box plots with mean value and standard error for each area. **B)** Distribution of the latencies of MEPs evoked from the two areas in each muscle recorded. **C)** Distribution of the latencies of MEPs evoked from the two areas with one-pulse stimulation (left) and multiple-pulse stimulation (right) in subsample A (oro-facial) and B (hand-arm). In order to show the distribution at population level the latencies in milliseconds were standardized in Z score within each patient for each muscle independently by area. The dashed line coincides with the normal distribution of the MEP latencies expressed in Z score. In each figure the right-upper panel shows a box plots with mean value and standard error each area.

From *Fornia et al., 2016 modified*.

representation not analysed here) confirmed the well-known homunculus with a dorsal region hosting the hand-arm representation and in a ventral region located at the posterior functional border with the hand-arm and oro-hand region of vPM, hosting the oro-facial representation. The oro-facial representations within M1 and vPM represented the starting sites for DES stimulation during speech tasks.

Comparison of the DES-induced interference effects in M1, vPM and Broca's area

The analysis on LF-DES-induced interference effects in three different areas was performed because the preliminary study on healthy subject, by means of Mixed-effects model (see Materials and Methods section), demonstrated that repeating two different words the same amount of times (in our case, 5 times) and comparing a frequency in the spectrum given the same muscle, the two logarithmic frequency means are not significantly different and it is not possible to distinguish between the two words (see **BOX 3** for statistical details). At physiological level, this result means that the force exerted by phono-articulatory muscles to denominate different words is comparable.
This result is in preparation for an additional publication.

DES-induced interference effects during task performance

Based on the results discussed above, only vPM and M1 can be strictly considered motor areas, hosting oro-facial and hand-arm muscles representation, while from Broca's area no motor output was observed, neither MEPs nor impairments of pre-activated muscles were recorded, suggesting that Broca's area, in contrast to recent studies (Di Pellegrino et al., 1992; Rizzolatti et al., 1996), cannot be considered a motor area. However, despite some criticism (Flinker et al., 2015), for many years, Broca's area has been considered a cortical area involved in motor control of speech.
The lack of motor output in resting and pre-contracted condition does not exclude per se a possible action on this area on motor apparatus exerted when the area is activated in ecological conditions. To shed light of this issue, the starting premise of this project was that, if Broca's is involved in motor control of speech, its temporary inactivation would induce a significant alteration of the phono-articulatory activity when compared with the *natural* speech *performance*. Thus, we decided to investigate the effect of LF-DES applied on Broca's area, besides to vPM and M1, during task performance. However, while stimulation of vPM and M1 was initially applied over the oro-facial

representations previously localized (**Fig. 3.4**), the stimulation of Broca's area was applied on the whole BA44/45 surface.

BOX 3. Mixed-effects model

In our problem, we defined the following mixed model:

$$y_{ijkl} = \mu + a_i + \beta_j + p_{ik} + \varepsilon_{ijkl} \qquad (1)$$

where, i is the index for subjects, j is the index for muscles, k is the index for words and l is the index for repetitions. $a_i \sim N(0, \sigma^2_a)$ is random effect of subject i, β_j is the fixed effects of muscle j, $p_{ik} \sim N(0, \sigma^2_p)$ is the random effect of word k and $\varepsilon_{ijkl} \sim N(0, \sigma^2)$ are random errors.

This formulation allows considering subjects as block factor, with effect a_i. Within each block is possible to measure the effect of words.

We considered also the simple model:

$$y_{ijkl} = \mu + a_i + \beta_j + \varepsilon_{ijkl} \qquad (2)$$

which does not include the random effect of words. The difference between Model 1 and Model 2 represents the deviation from the mean signal (Model 2).

A logarithmic transformation of the y values has been done in order to have comparable measurements.

We estimated the models for each frequency of the power spectrum and we estimated the variance of words, σ^2_p, and the variance of repetitions or residual variance, σ^2_r.
We also performed the F-test with H_0: $\sigma^2_p = 0$ for each frequency. Given the results, we always refused H_0. In other words, the σ^2_p is significantly different from 0 in each frequency of the spectrum. However, σ^2_p is always smaller than σ^2_r.

It means that, for instance, repeating two different words the same amount of times and comparing a frequency in the spectrum given the same muscle, the two logarithmic frequency means will not be significantly different and it will not be possible to distinguish between the two words.

For this study, a subgroup of 58 patients were considered: in 20 patients the DES was applied on Broca's area, in 45 on vPM and in 10 on M1, during object picture naming and/or counting tasks. In all patients, the dominant hemisphere for language was the left one, and the stimulation paradigm adopted was the LF-DES. M1 or vPM were stimulated at Threshold Intensity, *(ThreshI*-LF-DES*)*, i.e. the minimum intensity needed to induce a consistent interference on the task performance; Broca's area was initially stimulated with the same intensity, then the intensity was increased until a clear effect on speech performance was obtained *(SupraThreshI*-LF-DES*)*. The intensity value (average stimulation intensity ± standard deviation -SD-) applied onto M1 was 3.00 ± 1.15 mA for a train duration (average train duration ± SD) of 2.43 ± 1.36 sec. The intensity value applied onto vPM was

2.75 ± 0.93 mA, for a train duration of 2.57 ± 1.02 sec. The intensity value applied onto Broca's area was increased to 3.45 ± 1.3 mA, because only with higher current intensity, the LF-DES interfered with speech tasks, and the train duration was 2.57 ± 1.02 sec.
The complex clinical setting and the primary aim to avoid any impact on the clinical procedure time, did not allow of collection the same number of stimulations in each patient.

Broca's area. In 20 patients (M = 13 and F = 7; 37±12 years) the LF-DES was applied onto Broca's area during both object picture naming and counting tasks (16 and 7 patients respectively). The total number of recorded trials was 97, while the mean number of trials for subject (± SD) was 4.85 ± 3.79, independently of the speech tasks.

vPM. In 45 patients (M = 29 and F = 16; 39±15 years) the LF-DES was applied onto vPM during object picture naming and/or counting tasks (26 and 29 patients respectively). The total number of recorded trials was 226, while the mean number of trials for subject (± SD) was 5.02 ± 1.81, independently of the speech tasks.

M1. In 10 patients (M = 5 and F = 5; 42±7 years) the LF-DES was applied onto M1 during both object picture naming and counting tasks (7 and 10 patients respectively). The total number of recorded trials was 51, while the mean number of trials for subject (±SD) was 5.10 ± 2.69, independently of the speech tasks.

Clinical inspection of the functional outcome

The first performed analysis concerned the classification of the vocal effects induced by the LF-DES on cortices, while patients performed speech tasks.

*Broca's area.*When the *ThreshI*-LF-DES was applied onto Broca's area, during both speech tasks, it failed to induce a clinically apparent impairment of speech: neither anarthria/speech arrest and dysarthria nor an interruption of speech performance were observed: the patients correctly performed the task, naming the presented pictures or counting. Notably, the same *ThreshI*-LF-DES failed to induce any effect on speech performance in all patients (20 patients, 100%). The absence of an interference effect of the *ThreshI*-LF-DES was observed both at motor level and at semantic and phonological level for tasks composed by single words. However, it is reported in the neurosurgical literature that the LF-DES applied onto Broca's area actually impairs the speech performance inducing the speech arrest effect (Luders et al., 1987; Axelson et al., 2009; Chang et al., 2011, 2016; Tate et al., 2014; Sarubbo et al., 2016), therefore, according to clinical needs, the current intensity of the LF-DES was increased until the stimulus induced speech arrest. The increased intensity was defined

SupraThreshI-LF-DES, since it was always higher (4,3±1,3 mA) than *ThreshI*-LF-DES established stimulating vPM (2.75 ± 0.93 mA for vPM) and/or M1 (3.00 ± 1.15 mA for M1) (see above).
The speech arrest with *SupraThreshI*-LF-DES, was observed only if the current was applied over the ventral and posterior portion of Broca's area (ventral BA44, vBA44) and if it was applied in the interval between the object presentation and the word articulation or between 2 subsequent numbers. During DES stimulation the patients remain silent without any attempt to pronounce the word (either a name or a number) and their phono-articulatory muscles, expected to activate for the articulation of the word to be pronounced, remain at rest and the background EMG activity is not affected. When the stimulation is removed, the motor activation is performed and the name correctly pronounced. This effect was found in all patients stimulated with a *SupraThreshI*-LF-DES (7 out of 20, 100%). If, instead, the *SupraThreshI*-LF-DES was applied on vBA44 when the word articulation had already begun, it failed to induce an impairment of speech. This condition was assessed in only 2 out of 7 patients, in both showing the same effect. *SupraThreshI*-LF-DES applied on other sectors of Broca's area beyond vBA44, failed, in all patients (N=7) to induce an impairment of the speech both at motor level and at semantic and phonological level, suggesting that Broca's area does not exert a role in motor control of speech and neither in semantic and phonological constructions of single words.

vPM. When vPM was stimulated with *ThreshI*-LF-DES, it induced in all patients (45 patients, 100%) different functional impairments task performance (either naming or counting), although all the stimulus duration. The interferences were vocalizations-like effects or dys-phono-articulations. In the first case, the patient, during the stimulation, kept vocalizing the first vowel of the word/number to be pronounced, while in the second the patients did not articulate correctly the word, despite trying to with a clear impairment of vocal emission with respect to natural performance. This dys-phono-articulatory interference was the most commonly observed functional outcome upon application of LF-DES onto vPM (83% of the interferences were dys-phono-articulations while the 17% of the interferences were vocalizations-like effects). Similar phenomena were described by Penfield and Boldrey (1937, in a similar setting). However, the most recent neurosurgical literature (Petrovich et al., 2005; Chang et al, 2011,2016; Matsuda et al, 2014; Tate et al, 2014; Mandonnet et al, 2016), reports different observation claimimg that the LF-DES applied to vPM induced a proper anarthria/speech arrest effect, defined as the arrest of ongoing speech in absence of vocal output and simultaneously absence of motor responses, an effect never observed in our patients (100%) when stimulating this area.

M1. *ThreshI*-LF-DES stimulation on the oro-facial representation of M1 induced a speech arrest, a phenomenon characterized by an absence of vocal output, due to the visible tonic contraction of several phono-articulatory muscles, making the articulation of words impossible for the whole duration of the stimulation. When the stimulus was removed,, the phono-articulatory function was immediately restored and the patients denominated correctly the word. The stimulus was applied onto M1 during both tasks and induced this effect on all stimulated (100%) sites in all patients (10 patients, 100%). The same effect was reported by Tate and colleagues (2014) and by Deletis and colleagues (2014), the latter actually suggesting that the contraction was "presumably" accompanied with dysphonic/aphonic speech, not described in other studies. We here report an absence of vocal output in 100% of cases.

Qualitative analysis of the speech interference of the EMG signals

Aside of the qualitative analysis of the performance output of the patients reported by clinical inspection/evaluation, we report the qualitative analysis of the EMG activity of the clinically recorded phono-articulatory muscles (OO, MENT and MYLO contra- and ipsilateral, and contralateral PLATYSM, when recorded) during the LF-DES interferences on three areas.

Broca's area. When *ThreshI*-LF-DES was applied onto Broca's area, it did not affect the performance, and the EMG activity pattern during the stimulation seemed, at inspection, superimposable to the EMG activity pattern of the same muscles in the *natural performance* (**Fig. 3.7**). When *SupraThreshI*-LF-DES was applied before words articulation, the speech arrest occurred and the EMG activity pattern seemed superimposable to the background EMG recorded in the same muscles (**Fig. 3.8).**

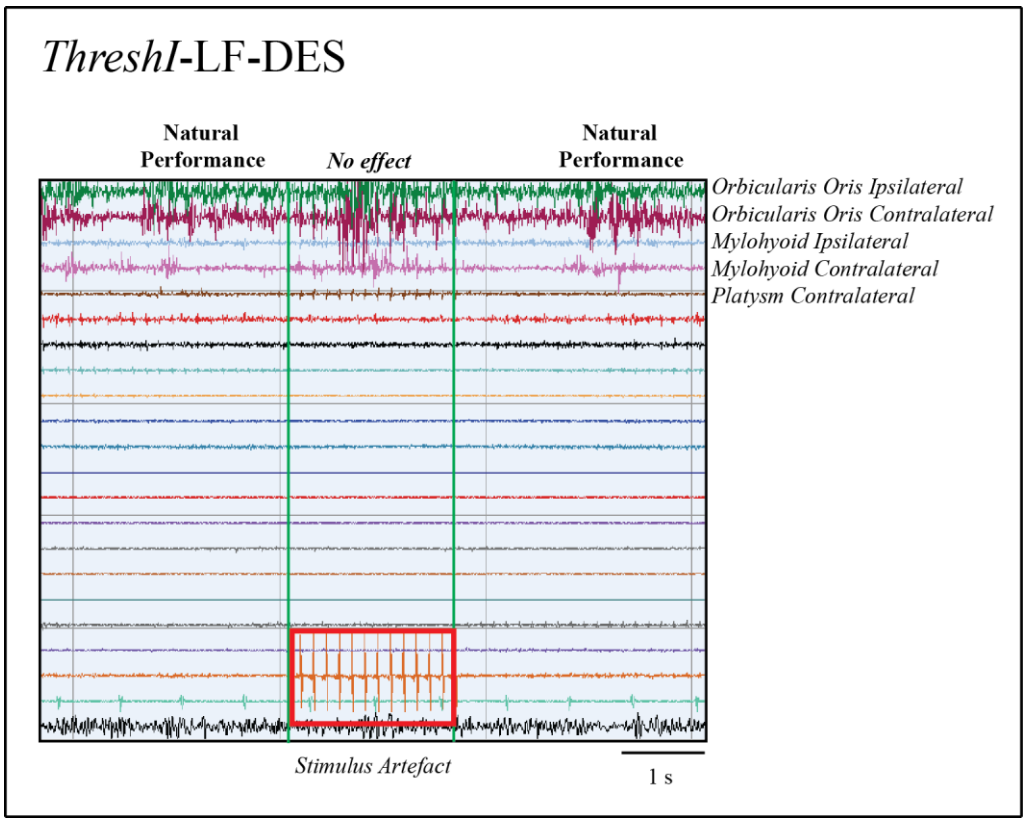

Figure 3.7 Example of patient in which Broca's area was stimulated by the LF-DES during counting task. The EMG trace represents i) the natural speech performance without the stimulation, ii) the time of the stimulation of Broca's area with the *ThreshI*-DES (4mA, stimulus artefact represented in the red rectangle on muscle channel) and ii) again the natural performance. The *ThreshI*-DES of Broca's area seems not to interfere with speech production. The EMG signal during the LF-DES appears similar to that of the natural performance.
On the right, the phono-articulatory recorded muscles are indicated. The stimulus artefact is also represented in the red rectangle on a muscle channel.

vPM. When *ThreshI*-LF-DES was applied on vPM, it impaired the vocal-related outcome of patients inducing vocalization-like and dys-phono-articulations. At a first qualitative analysis, the EMG activity showed three different patterns.

The first EMG pattern occurred in 11 patients (24,4%) and was characterized, both in object picture naming and in counting task, by a rhythmic *Clonic-like interference* in the activity of phono-articulatory recorded muscles: during stimulation the ongoing activity of muscles switched to a *Clonic-like* pattern. It was observed in 7 patients during counting and in 7 during naming task (**Fig. 3.9**).

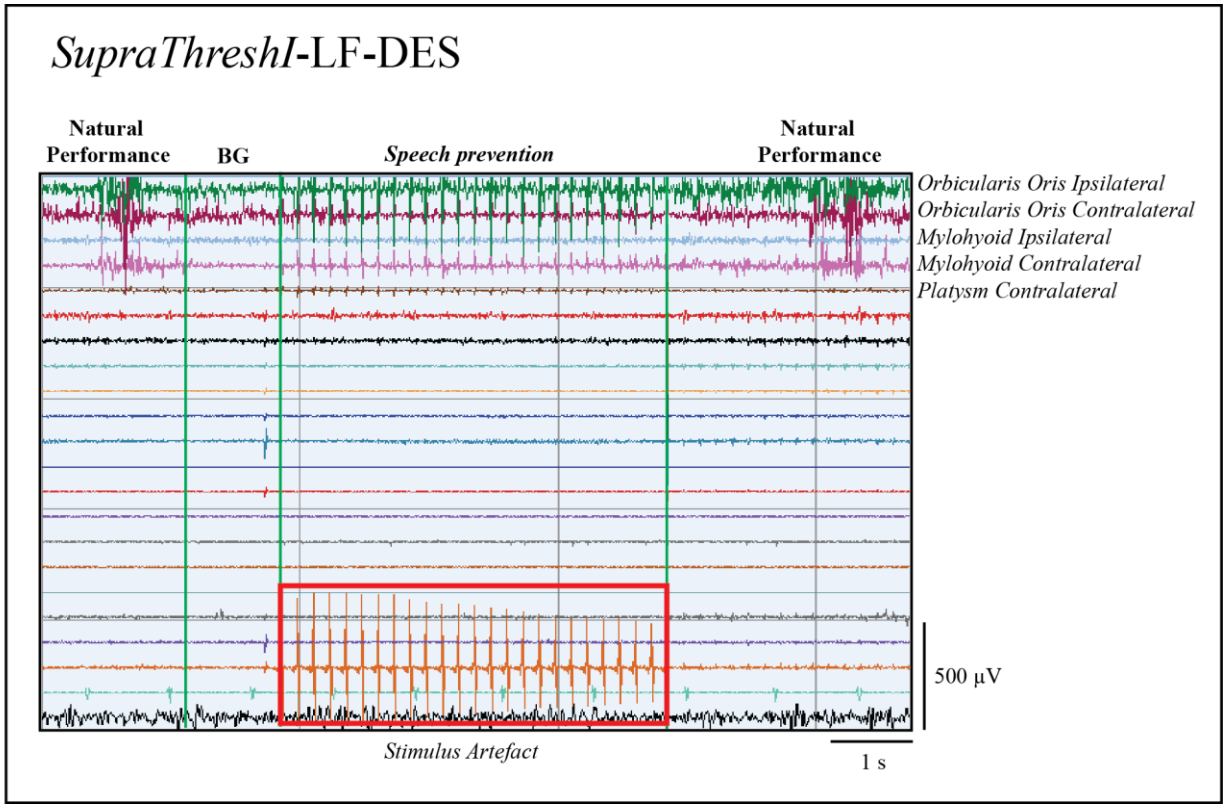

Figure 3.8 The patient of this EMG trace is the same patient of that in figure 3.7. The EMG trace represents i) the natural performance, ii) the background signal (BG, resting state condition), iii) the time of the stimulation of Broca's area with the *SupraThreshI*-DES (6mA, stimulus artefact represented in the red rectangle on muscle channel) and ii) the natural performance after the removal of the stimulus. The *SupraThreshI*-DES of Broca's area seems prevents the speech production. The EMG signal during the LF-DES appears similar to that of the background (BG) and not to that of the natural performance.
At right, the phono-articulatory recorded muscles are indicated. When the stimulus was applied over the cortex, in some muscle channels (phono-articulatory muscles, see first channels) was recorded the stimulus artefact. The stimulus artefact is also represented in the red rectangle on a muscle channel.

In 60% of the patients, this pattern was associated with dys-phono-articulations and in 40% with vocalizations-like effects. The absence of any simultaneous sign of epileptic activity in the ECoG recording of all patients reasonably excluded the possibility that this pattern might have been the expression of seizure.

The second EMG pattern occurred in 14 patients (31,1%) and was characterized by an *Pure Inhibitory interference* on phono-articulatory muscles, independently from the task being performed: during stimulation the ongoing activity of the muscle activated in natural performance was inhibited. it was observed in 8 patients during counting and in 9 during object picture naming task (**Fig. 3.10**). This

pattern was mostly associated with dys-phono-articulations (85.7%) rather than vocalizations-like effects (14.3%).

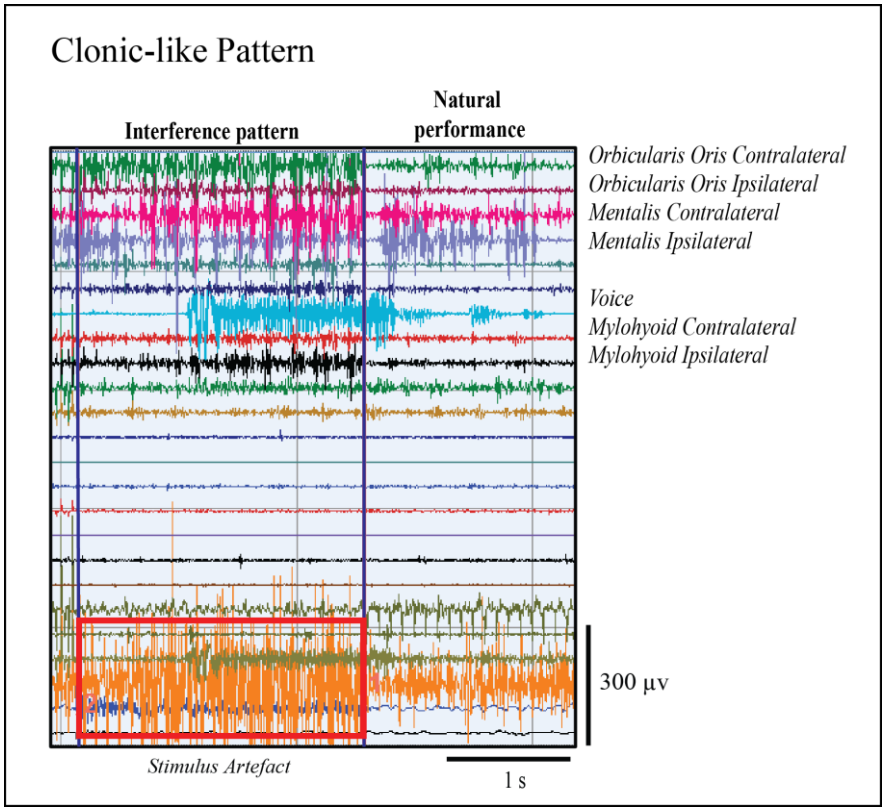

Figure 3.9 The EMG trace represents the interference *Clonic-like* pattern and the natural performance. At right, the phono-articulatory recorded muscles are indicated. When the stimulus was applied over the cortex, in some muscle channels (phono-articulatory muscles, see first channels) was recorded the stimulus artefact. The stimulus artefact is also represented in the red rectangle on a muscle channel.

The third EMG pattern occurred in 23 patients (51,1%) and was characterized by a simultaneous *Inhibitory/Recruiting* action on the phono-articulatory muscles, independently from the task performed: during stimulation some of the activated muscles were inhibited while others were recruited. It was observed in 17 patients during counting and in 14 during object picture naming task (**Fig. 3.11**). Again, this pattern was mostly associated with dys-phono-articulations (85.7%) rather than vocalizations-like effects (14.3%).

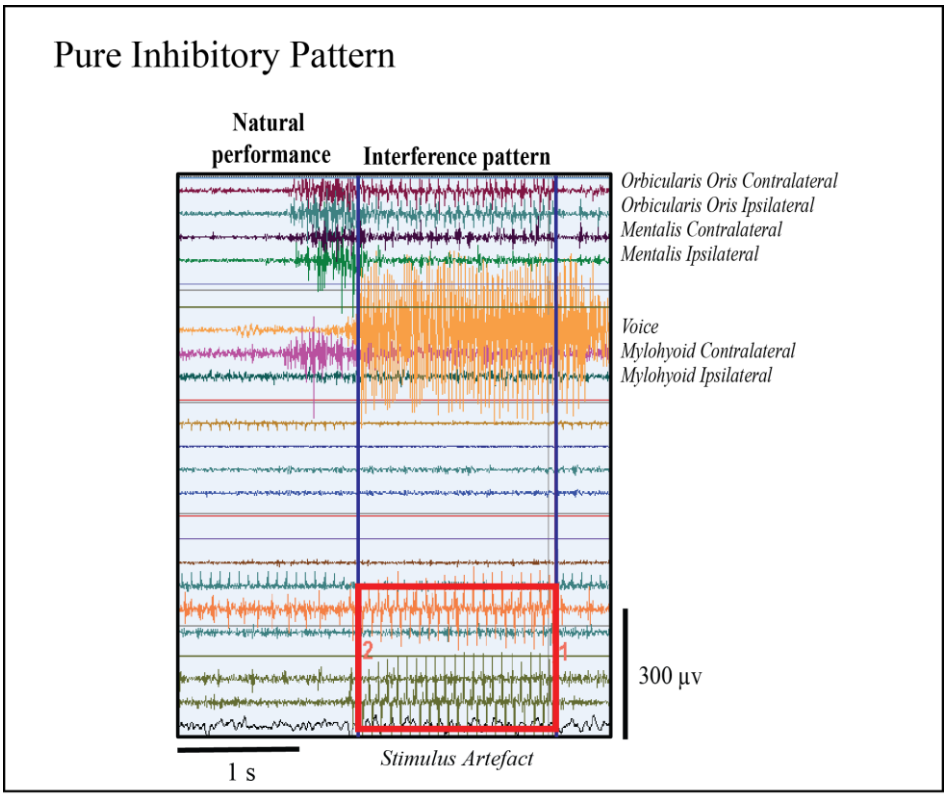

Figure 3.10
The EMG trace represents the natural performance and the interference *Pure Inhibitory* pattern.
On the right, the phono-articulatory muscles recorded are named. When the stimulus was applied over the cortex, the stimulus artifact was recorded in some muscle channels (phono-articulatory muscles, see first channels). The stimulus artifact is represented in the red rectangle.

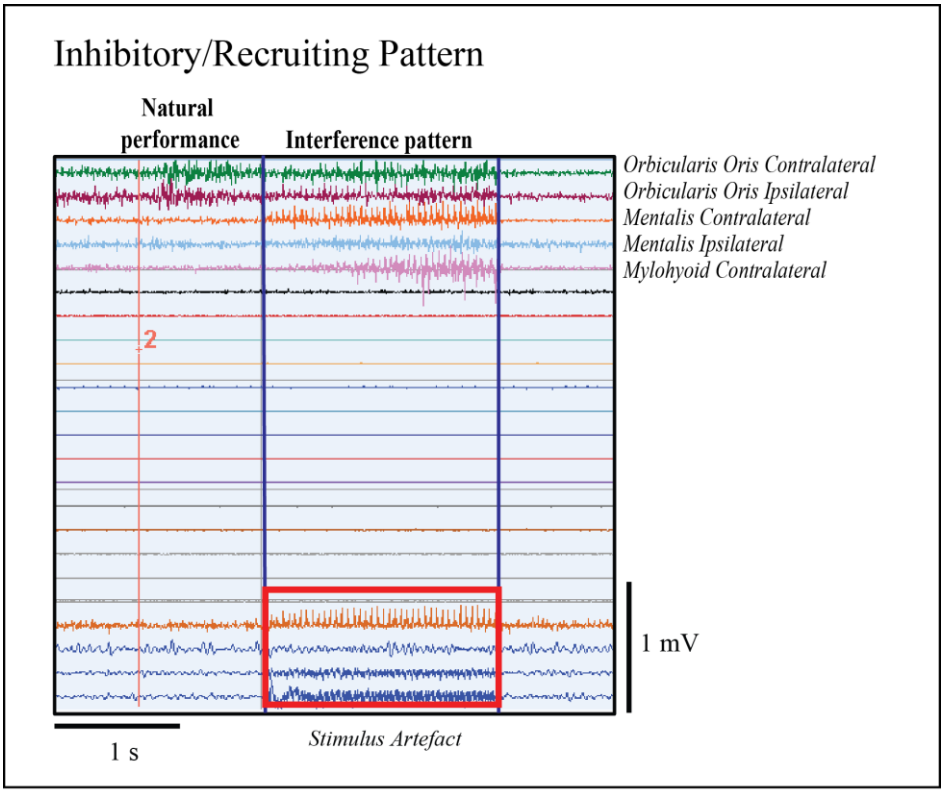

Figure 3.11
The EMG trace represents the natural performance and the interference *Inhibitory/Recruiting* pattern.
On the right, the phono-articulatory muscles recorded are named. When the stimulus was applied over the cortex, the stimulus artifact was recorded in some muscle channels (phono-articulatory muscles, see first channels). The stimulus artifact is represented in the red rectangle.

M1. *ThreshI*-LF-DES stimulation of the oro-facial representation of M1 impaired the vocal outcome of patients inducing a speech arrest phenomenon and was characterized by a clear tetanic contraction of different oro-facial muscles (**Fig. 3.12**) in 100% patients. This specific and widespread tonic muscle recruitment, due to the activation of the M1 oro-facial representation, reasonably prevented the adequate articulation of the words/numbers for the whole duration of the stimulation.

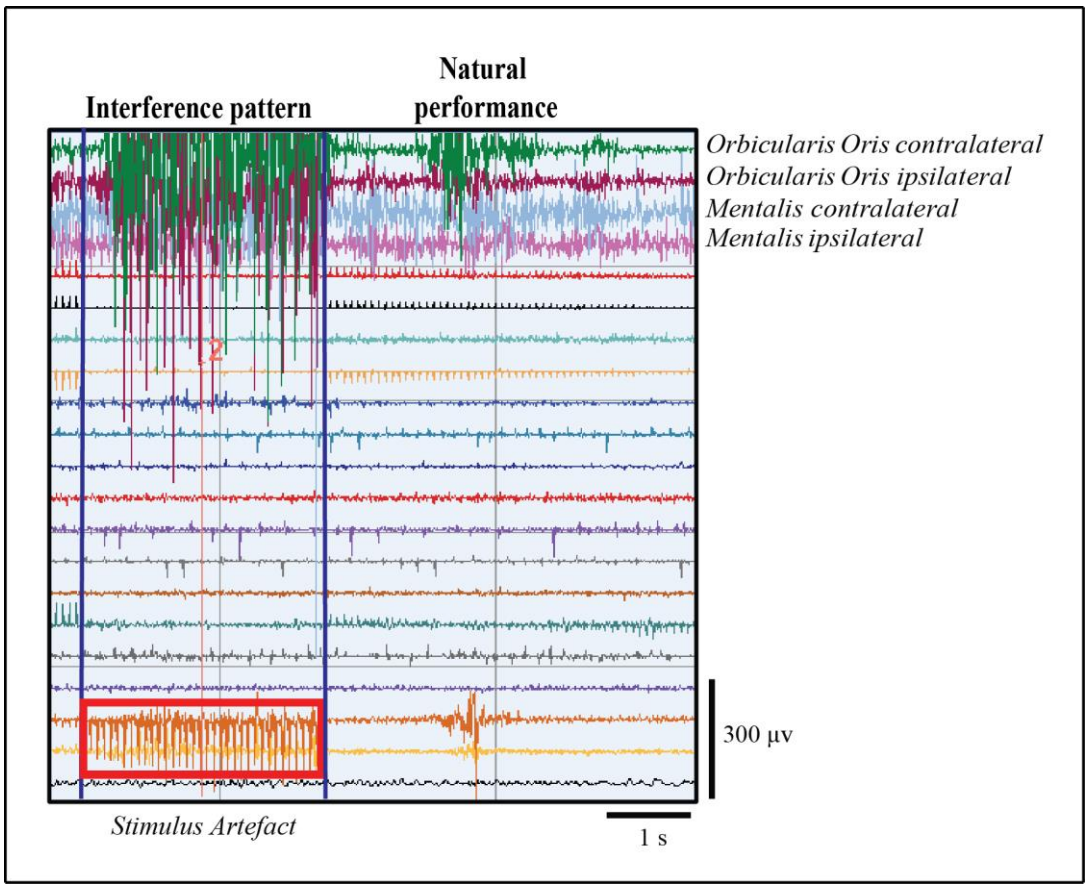

Figure 3.12 The EMG trace represents the interference pattern on activity of M1 during speech task and the natural performance.
On the right, the phono-articulatory recorded muscles are indicated. The stimulus artefact is represented in the red rectangle.

Quantitative analysis of the EMG signals corresponding to performance during the LF-DES

Following the qualitative analyses, a quantitative analysis of the EMG signals of the recorded muscles was performed to support, with a quantitative measure and a statistical analysis, the qualitative analysis performed by visual inspection and to add new elements on the possible effect of a transient lesion of the three areas on muscle activity during task performance. The analysis applied on EMG signals allowed indeed to infer, and then compare, the motor unit recruitment of the phono-

articulatory recorded muscles in two different situations: the *natural performance* and the performance during the application of the LF-DES on the areas of interest (*LF-DES interference*), considering separately naming and counting tasks. The quantitative analysis of the EMG signals in both conditions was performed in time and in frequency domain by calculating the mean and the peak values of the Root Mean Square (RMS) and the mean and median frequency and area values of the Power Spectrum (PS) of each analysed EMG trace (see Materials and Methods section).

As defined in Materials and Methods section, ideally the two conditions (*LF-DES interference vs natural performance*) should be compared when the patient denominates the same word (object or number). However, the clinical setting did not allow performing this procedure (only in 2 out of 58 patients). Thus, the Correlation Coefficient (CC) was used, in each patients and for each muscle, to compare the EMG signal related to different words, measured by calculating the PSs curves related to different words either objects or number considered separately. 31 patients performed counting task, while 29 performed object picture naming task.

	Counting							**Object picture Naming**					
	OO i	OO c	Mylo c	Mylo i	Ment c	Ment i		OO i	OO c	Mylo c	Mylo i	Ment c	Ment i
Mean	0,87	0,88	0,91	0,90	0,88	0,88		0,88	0,88	0,91	0,92	0,89	0,90
SD	0,06	0,06	0,05	0,05	0,07	0,07		0,04	0,04	0,04	0,03	0,04	0,04

Population analysis. I = ipsilateral; C = Contralateral; SD = Standard Deviation.

CC >0.7 indicates a significantly strong correlation. In all our cases, the CCs was higher than 0.7, almost closest to 1, therefore the different words pronounced by each patients and for each muscle, are almost superimposable. This data suggest that independently from articulated word, the recorded muscles exert an analogous pattern of activation in phono-articulation. This result allowed to investigate the effect of LF-DES applied to Broca's area, vPM and M1 during task performance, independently from word denominated from patient. This result has been confirmed in two cases in which was possible comparing the PSs curves related to same muscle and same word: the CC values were indeed similar to those reported in table above.

Broca's area

ThreshI-LF-DES. By comparing in each patient, by means of Mann Whitney test, each RMS and PS parameter calculated in *natural performance* and during the application of the *ThreshI*-LF-DES (mean intensity 2.7±0.7 mA; train duration 2.8±1.0sec) onto Broca's area, it emerged that in all patients (100%, 20 patients) no significant differences were found ($p>.05$) between EMG signals related to speech tasks with and without stimulation: the mean and median frequencies and the area of PSs were not significantly different in the two conditions ($p>.05$); the mean and peak values of RMSs of EMG signals were not significantly different ($p>.05$) in two conditions (**Fig. 3.13 A**). In all patients, the p values are more similar to 1 in comparison with 0.05. These results were obtained for each phono-articulatory recorded muscle and in both speech tasks (in 16 patients the *ThreshI*-LF-DES was applied during object picture naming and in 7 during counting). These data suggest that the *ThreshI*-LF-DES onto Broca's area is ineffective in inducing any significant effect on motor unit recruitment recorded in speech production. This quantitative analysis fully supports the functional outcome, characterized by a lack of any deficit in phono-articulation (neither anarthria or dysarthria or interruption of speech performance observed), and with the qualitative observation of EMG traces.

SupraThreshI-LF-DES. For clinical needs, in 7 out of 20 patients, the intensity of the LF-DES was increased from 2.7±0.7 mA (*ThreshI*-LF-DES) to 4.3±1.3 mA (*SupraThreshI*-LF-DES), resulting in the speech arrest. This effect was reliably obtained when the stimulation was applied on the ventral and posterior part of Broca's area (vBA44). By comparing in each patient, with Mann Whitney test, each RMS and PS parameter calculated in each muscle during *natural performance* and during *SupraThreshI*- DES, it emerged that in all patients (100%) significant differences were found ($p<.05$): the mean and median frequencies and the area of PSs were significantly different ($p<.05$); the mean and peak values of RMSs were significantly different ($p<.05$). In all patients, the p values are more similar to 0 in comparison with 0.05. These results were obtained in both tasks. Interestingly, based on this EMG analysis and on the clinical observation reporting a complete lack of attempt in speech production during stimulation, an additional analysis was performed in order to investigate whether the stimulation completely prevented the motor output or whether it inhibited the muscle ongoing activity. In the first case, the EMG signal recorded during the stimulation would be superimposable to the EMG signal recorded in resting state, i.e. to the background EMG (BG-EMG), for all recorded muscles, suggesting a complete lack of onset of motor recruitment. In the second case, the EMG signal recorded during the stimulation would be inhibited when compared to *natural performance*, and, consequently would not be superimposable to BG-EMG. By comparing in each patient (7), by means of Mann Whitney test, each RMS and PS parameter calculated during the application of the *SupraThreshI*-LF-DES onto vBA44 with that calculated at resting state (BG-EMG), it emerged that in all patients (100%, 7 patients) no significant differences were found ($p>.05$): the

mean and median frequencies and the area of PSs were not significantly different (p>.05); the mean and peak values of RMSs of EMG signals in two conditions were not significantly different (p>.05) (**Fig. 3.13 B**). These results were obtained for each phono-articulatory recorded muscle, in both speech tasks (in 5 patients the *SupraThreshI*-LF-DES was applied during object picture naming and in 2 during counting). The muscle activity occurring during *SupraThreshI*-LF-DES stimulation of vBA44 was indeed superimposable with the background muscle activity (BG-EMG), suggesting that the stimulation actually prevents the onset of the motor output (speech *prevention*) rather than stopping/impairing its ongoing execution (speech *arrest*), as reported in neurosurgical literature (Tate et al., 2014; Fujii et al., 2015).

2 patients were stimulated when the speech production had already begun. In these cases, the *SupraThreshI*-LF-DES, applied in the same sites eliciting the *speech prevention* phenomenon, was delivered before the onset of word phono-articulation, and it failed to induce any effect: the patients denominated correctly the word/number and no significant differences were found (p>.05) between the stimulation (*LF-DES interference*) and the *natural performance* conditions: the mean and median frequencies and the area of PSs were not significantly different (p>.05); the mean and peak values of RMSs were not significantly different (p>.05). This result suggest that once the phono-articulatory process has started, the impairment of Broca's area does not affect the ongoing motor activity, challenging the role of Broca's area in controlling the motor output of speech production. Should this be infect the case, the stimulation would induce a prevention/arrest of the ongoing activity when delivered before and during the denomination.

The observation that the effect of the LF-DES onto Broca's area is intensity-related raised the question of whether the higher intensity was actually acting on neurons hosted/located in the cortical layer of Broca's area or rather, by spread of current, reached subcortical fibres running below Broca's area at the stimulated site and thus inducing the effect. In 2 patients, the *SupraThreshI*-LF-DES was delivered to the subcortical fibres proximal to the cortical region of Broca's area inducing the *speech prevention* phenomenon. The effect of subcortical stimulation was in all-similar to the effect obtained at cortical level with the *SupraThreshI*-LF-DES, the patients did not articulate the words/numbers and the EMG signal recorded during the stimulus was not significantly different (p>.05) with respect to the BG-EMG: in both patients the mean and median frequencies and the area of PSs were not significantly different (p>.05) in the two conditions as well as the mean and peak values of RMSs.

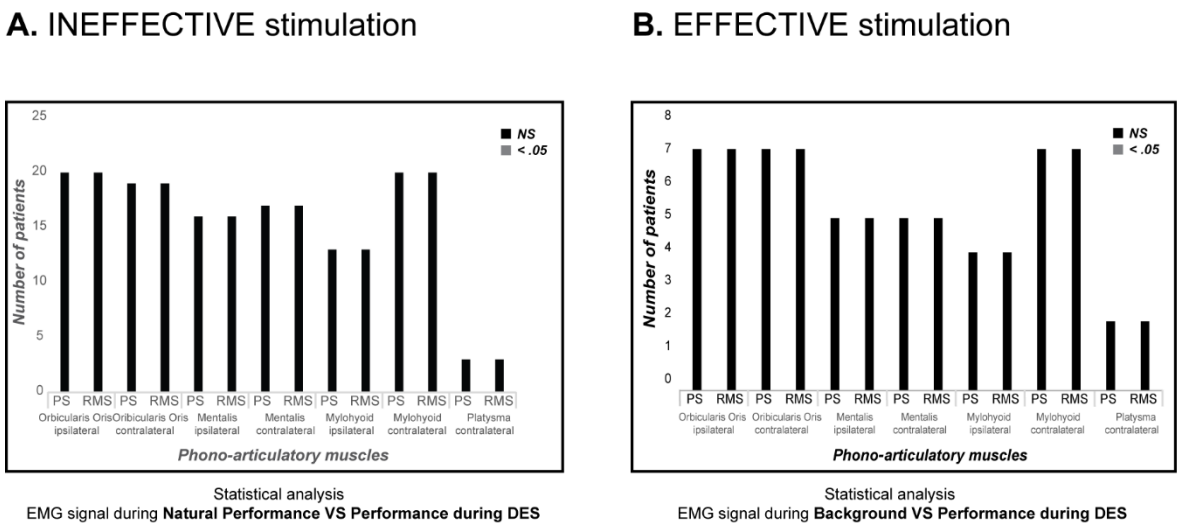

Figure 3.13 Statistical analysis: **A)** the chart shows for each recorded muscle in all 20 patients if there is a significantly difference (Mann Whitney U-test) between each PS and RMS parameters in natural performance *vs* performance during *ThreshI*-DES. Black bars indicate that there is Not a Significantly (NS) difference between two conditions. Instead, the grey bar would indicate a significantly different ($p<.05$). The *ThreshI*-DES of Broca's area does not interfere with speech production. **B)** The chart shows for each recorded muscle in 7 patients in which was applied the *SupraThreshI*-DES, if there is a significantly difference (Mann Whitney U-test) between each PS and RMS parameters in background signal *vs* performance during *SupraThreshI*-DES. Black bars indicate that there is Not a Significantly (NS) difference between two conditions. Instead, the grey bar would indicate a significantly different ($p<.05$). The *SupraThreshI*-DES of Broca's prevents totally the speech production.
In both charts, it is evident that not in all patients are recorded seven phono-articulatory muscles.
PS: Power Spectrum, RMS: Root Mean Square.

vPM

Qualitative analyses suggested that vPM plays a role in motor programming of phono-articulatory muscles. Two different types of vocal interferences were induced by *ThreshI*-LF-DES while patients denominated the words: vocalizations-like and dys-phono-articulations. However, the EMG induced-patterns were three: *Clonic-like*, *Pure Inhibitory* and *Inhibitory/Recruiting* patterns. We applied to the EMG patterns induced by *ThreshI*-LF-DES on vPM during speech tasks, the same analyses adopted with Broca's area.

From the analysis, three different patterns of interference on EMG activity emerged:

Group 1, *Clonic-like* Pattern. In the first group (N = 11), clustered 8 patients: in 6 patients the *ThreshI*-LF-DES was applied during counting and in 4 during object picture naming task (**Fig. 3.14**). In the 2 remaining cases, the stimulus was not applied for at least three times during the same speech task. We observed that in all muscles almost all the calculated parameters increased statistically with respect to *natural performance*.

As shown in **Figure 3.14**:

- for what concerns the ipsilateral OO, 1/8 case showed a statistically significant ($p<.05$) decrease (grey bars in figure) of the median PS frequency, 1/8 case of the PS area, 1/8 case of the mean RMS; 1/8 case showed a statistically significant ($p<.05$) increase (black bars in figure) of the median PS frequency, 3/8 cases of the PSs area, 3/8 cases of the mean RMS, 2/8 cases of the peak RMS;
- for what concerns the contralateral OO, 1/8 case showed a statistically significant ($p<.05$) decrease of the mean PS frequency, 1/8 case of the median PS frequency; 3/8 cases showed a statistically significant ($p<.05$) increase of the PSs area, 3/8 cases of the mean RMS, 3/8 cases of the peak RMS;
- for what concerns the ipsilateral MENT, 1/8 case showed a statistically significant ($p<.05$) increase of the PS area, 1/8 of the mean RMS;
- for what concerns the contralateral MENT, 1/8 case showed a statistically significant ($p<.05$) increase of the of the PS area, 2/8 cases of the mean RMS, 2/8 cases of the peak RMS;
- for what concerns the ipsilateral MYLO, 2/8 cases showed a statistically significant ($p<.05$) increase of the PSs area, 3/8 cases of the mean RMS, 2/8 cases of the peak RMS;
- for what concerns the contralateral MYLO,1/8 case showed a statistically significant ($p<.05$) decrease of the mean PS frequency; 1/8 case showed a statistically significant ($p<.05$) increase of the mean PS frequency, 5/8 cases of the PSs area, 5/8 cases of the mean RMS, 4/8 cases of the peak RMS.

This result suggests that the transient interference of the vPM activity during the task resulted in an increase of the frequency of discharge of the motorneurons innervating the recorded muscles, the increased recruitment of an ongoing motor unit or the recruitment of larger units. Notably, in 3/8 patients the stimulation recruited phono-articulatory muscles previously not involved in natural speech, again with *Clonic-like* pattern.

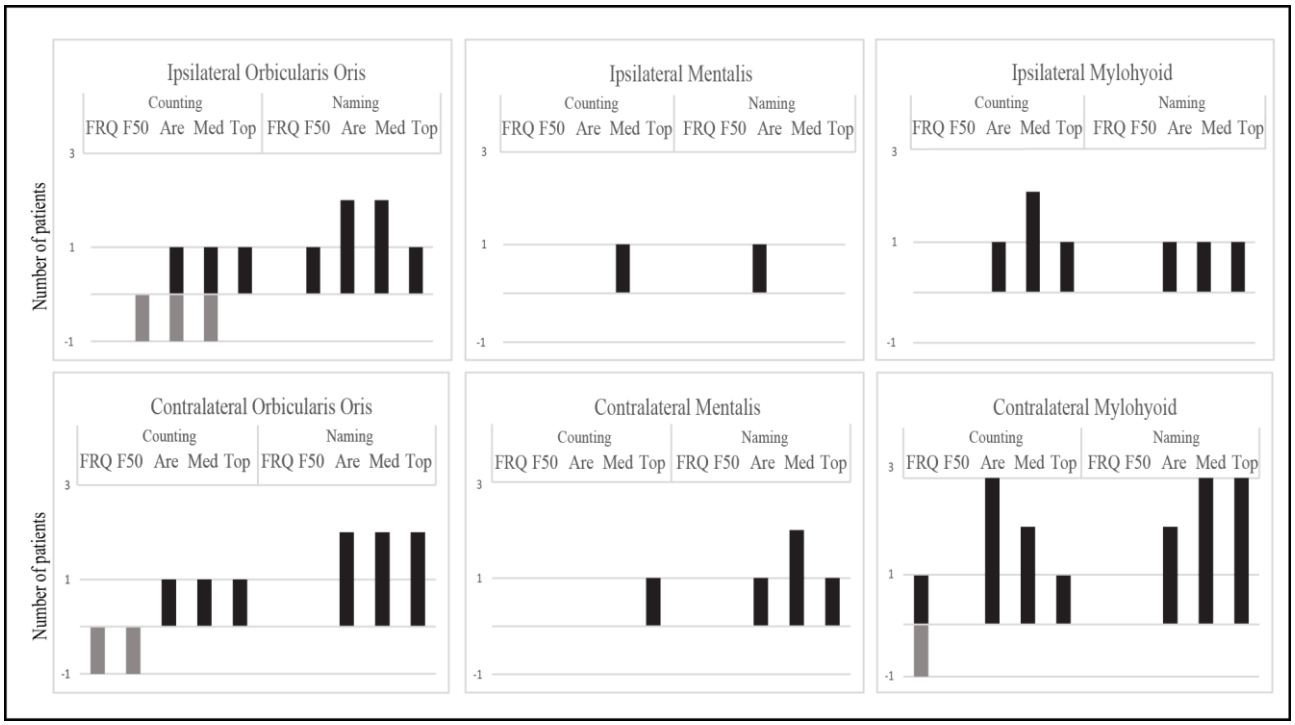

Figure 3.14 Statistical analysis on group 1: during speech tasks, the LF-DES induced a muscle *Clonic-like* recruitment. On the y-axis is the number of instances in which a statistically significant (p<.05) alteration of the values were induced by LF-DES, compared to natural performance. On the x-axis, the EMG-calculated parameters (FRQ = mean PS frequency, F50 = median PS frequency, Are= area under curve of PS, Med = mean RMS, Top = peak RMS) task-segregated are indicated. It's possible to appreciate how this group is characterized by a statistically significant increased of the EMG parameters.
PS: Power Spectrum, RMS: Root Mean Square.

Group 2, *Pure Inhibitory* Pattern. In the second group clustered 14 patients and the quantitative analysis was performed on 10: in 6 patients, the *ThreshI*-LF-DES was applied during counting and in 6 during object picture naming task (**Fig. 3.15**). In the remaining 4 cases, the stimulus was not applied for at least three times during the same task. Overall a statistically significant (p<.05) decrease (grey bars in figure) of the EMG parameters related to the interference were induced by LF-DES with respect to natural performance. As shown in **Figure 3.15**:

- for what concerns the ipsilateral OO, 3/10 cases showed a decrease of the mean PS frequency, 3/10 cases of the median PS frequency, 8/10 cases of the PSs area, 8/10 cases of the mean RMS, 8/10 cases of the peak RMS;
- for what concerns the contralateral OO, 1/10 case showed a decrease of the mean PS frequency, 4/10 of the median PS frequency, 9/10 of the PSs area, 9/10 of the mean RMS, 8/10 of the peak RMS;

- for what concerns the ipsilateral MENT, 3/10 cases showed a decrease of the mean PS frequency, 1/10 of the median PS frequency, 4/10 of the PSs area, 7/10 of the mean RMS, 5/10 of the peak RMS;
- for what concerns the contralateral MENT, 6/10 cases showed a decrease of the mean PS frequency, 4/10 of the median PS frequency, 4/10 of the PSs area, 8/10 of the mean RMS, 5/10 of the peak RMS;
- for what concerns the ipsilateral MYLO, 2/10 cases showed a decrease of the mean PS frequency, 2/10 of the median PS frequency, 3/10 of the PSs area, 3/10 of the mean RMS, 2/10 of the peak RMS;
- for what concerns the contralateral MYLO, 3/10 cases showed a decrease of the mean PS frequency, 5/10 of the median PS frequency, 3/10 of the PSs area, 2/10 of the mean RMS, 2/10 of the peak RMS.

This result suggest an actual pure inhibitory effect on the phono-articulatory muscles, induced by LF-DES, suggesting that the impairment of vPM activity results in a decrease of the frequency of discharge of the motor units and/or a decrease in the motor recruited units.

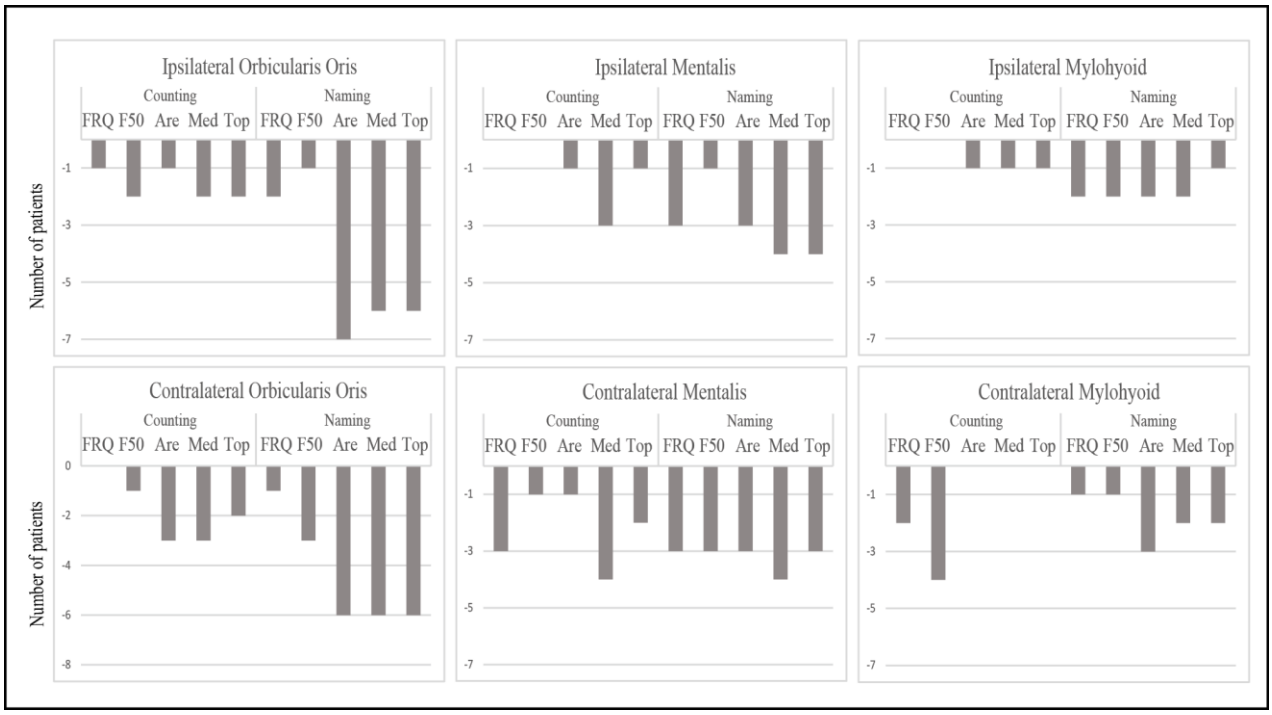

Figure 3.15 Statistical analysis on group 2: during speech tasks, the LF-DES induced muscle inhibition. On the y-axis is the number of instances in which a statistically significant ($p<.05$) alteration of the values were induced by LF-DES, compared to natural performance. On the x-axis, the EMG-calculated parameters (FRQ = mean PS frequency, F50 = median PS frequency, Are= area under curve of PS, Med = mean RMS, Top = peak RMS) task-segregated are indicated. It's possible to appreciate how this group is characterized by a statistically significant decreased of the EMG parameters.
PS: Power Spectrum, RMS: Root Mean Square.

Group 3, *Inhibition/Recruiting* Pattern. In the third group (N = 23), clustered 14 patients: in 11 patients, the *ThreshI*-LF-DES was applied during counting and in 5 during object picture naming task (**Fig. 3.16**). In the 9 remaining cases, the stimulus was not applied for at least three times with the same speech task. This was the most complex pattern of interference, showing a simultaneous inhibitory and excitatory effect on muscles by stimulating the same site. Interestingly in 5 (out of 14) patients not all recorded muscles were activated during natural speech performance, and the stimulus recruited muscles not physiologically active during performance.

As shown in **Figure 3.16**:

- for what concerns the ipsilateral OO, 1/14 case showed a statistically significant ($p<.05$) increase of the mean PS frequency (black bars) and 1/14 case of the peak RMS; 1/14 case showed a statistically significant ($p<.05$) decrease of the mean PS frequency (grey bars.), 4/14 of the median PS frequency, 8/14 of the PSs area, 11/14 of the mean RMS and 8/14 of the peak RMS;
- for what concerns the contralateral OO, 1/14 case showed a statistically significant ($p<.05$) increase of the mean PS frequency, 1/14 case of the PS area, 3/14 of the mean RMS; 2/14 cases showed a statistically significant ($p<.05$) decrease of the mean PS frequency, 4/14 of the median PS frequency, 4/14 of the PSs area, 8/14 of the mean RMS, 4/14 of the peak RMS;
- for what concerns the ipsilateral MENT, 3/14 cases showed a statistically significant ($p<.05$) increase of the PSs area and 1/14 of the mean RMS; 2/14 cases showed a statistically significant ($p<.05$) decrease of the mean PS frequency, 1/14 case of the median PS frequency, 2/14 cases of the mean RMS and 1/14 case of the peak RMS;
- for what concerns the contralateral MENT, 1/14 case showed a statistically significant ($p<.05$) increase of the PS area and 1/14 case of the peak RMS; 2/14 cases showed a statistically significant ($p<.05$) decrease of the mean PS frequency, 3/14 cases of the median PS frequency, 1/14 case of the PS area, 2/14 cases of the mean RMS and 2/14 cases of the peak RMS;
- for what concerns the ipsilateral MYLO, 1/14 case showed a statistically significant ($p<.05$) increase of the mean PS frequency, 4/14 cases of the PSs area, 3/14 cases of the mean RMS and 3/14 cases of the peak RMS;
- for what concerns the contralateral MYLO,1/14 case showed a statistically significant ($p<.05$) increase of the median PS frequency, 8/14 cases of the PSs area, 1/14 case of the mean RMS, 4/14 cases of the peak RMS; 2/14 cases showed a statistically significant ($p<.05$) decrease of the mean PS frequency, 1/14 case of the median PS frequency, 3/14 cases of the PSs area, 2/14 cases of the mean RMS and 3/14 cases of the peak RMS.

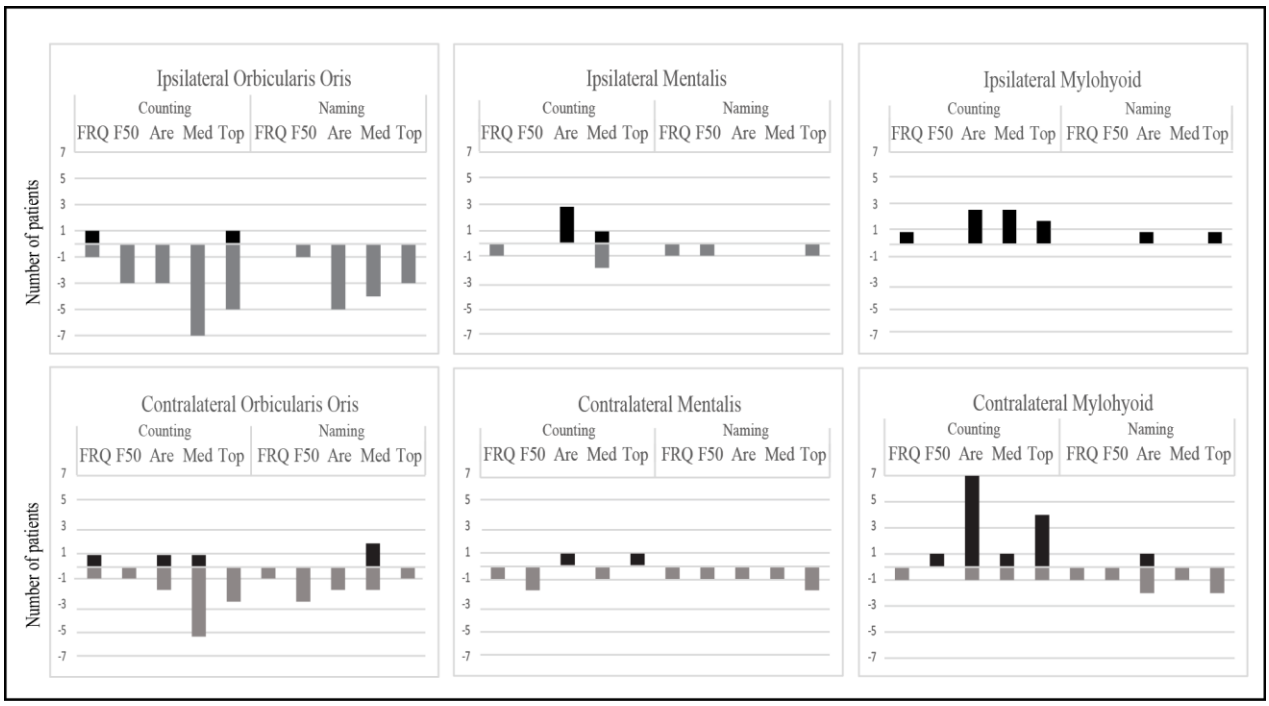

Figure 3.16 Statistical analysis on group 3: during speech tasks, the LF-DES induced a muscle inhibition and recruitment. On the y-axis is the number of instances in which a statistically significant ($p<.05$) alteration of the values were induced by LF-DES, compared to natural performance. On the x-axis, the EMG-calculated parameters (FRQ = mean PS frequency, F50 = median PS frequency, are= Area under curve of PS, Med = mean RMS, Top = peak RMS) task-segregated are indicated.
PS: Power Spectrum, RMS: Root Mean Square

M1

The *ThreshI*-LF-DES onto M1 induced a widespread tetanic recruitment of the phono-articulatory recorded muscles (**Fig. 3.12**) and, consequently an arrest of vocal output. The quantitative analysis, was performed on 7 patients (out of 10), since in the cases remaining, the stimulus was not applied for at least three times during the same task. In 2 patients, the *ThreshI*-LF-DES was applied during counting and in 5 during object picture naming task. The analysis showed, in **Figure 3.17**, that in all patients the EMG parameters increased significantly ($p>.05$) during stimulation:

- for what concerns the ipsilateral OO, 3/7 cases showed a statistically significant ($p<.05$) increase of the PSs area (black bars), 3/7 cases of the med RMS and 2/7 of the peak RMS;
- for what concerns the contralateral OO, 4/7 cases showed a statistically significant ($p<.05$) increase of the PSs area, 4/7 cases of the mean PS and 3/7 of the peak RMS;
- for what concerns the contralateral MENT, 1/7 case showed a statistically significant ($p<.05$) increase of the mean PS frequency, 1/7 case of the median frequency, 3/7 cases of the PSs area, 2/7 cases of the mean RMS and 2/7 cases of the peak RMS;

- for what concerns the ipsilateral MYLO, 2/7 cases showed a statistically significant ($p<.05$) increase of the PSs area, 2/7 cases of the mean RMS and 2/7 cases of the peak RMS;
- for what concerns the contralateral MYLO, 2/7 cases showed a statistically significant ($p<.05$) increase of the mean PS frequency, 2/7 cases of the median PS frequency, 7/7 cases of the PSs area, 7/7 cases of the mean RMS and 4/7 cases of the peak RMS.

At physiological level, the electrical stimulation of M1, by exciting pyramidal cells, induces the tonic recruitment of motor units, altering the temporal pattern of muscle activation needed to accomplish the proper articulation. Among analysed muscles in interferences, only the activity of the ipsilateral *mentalis* was not impaired by LF-DES.

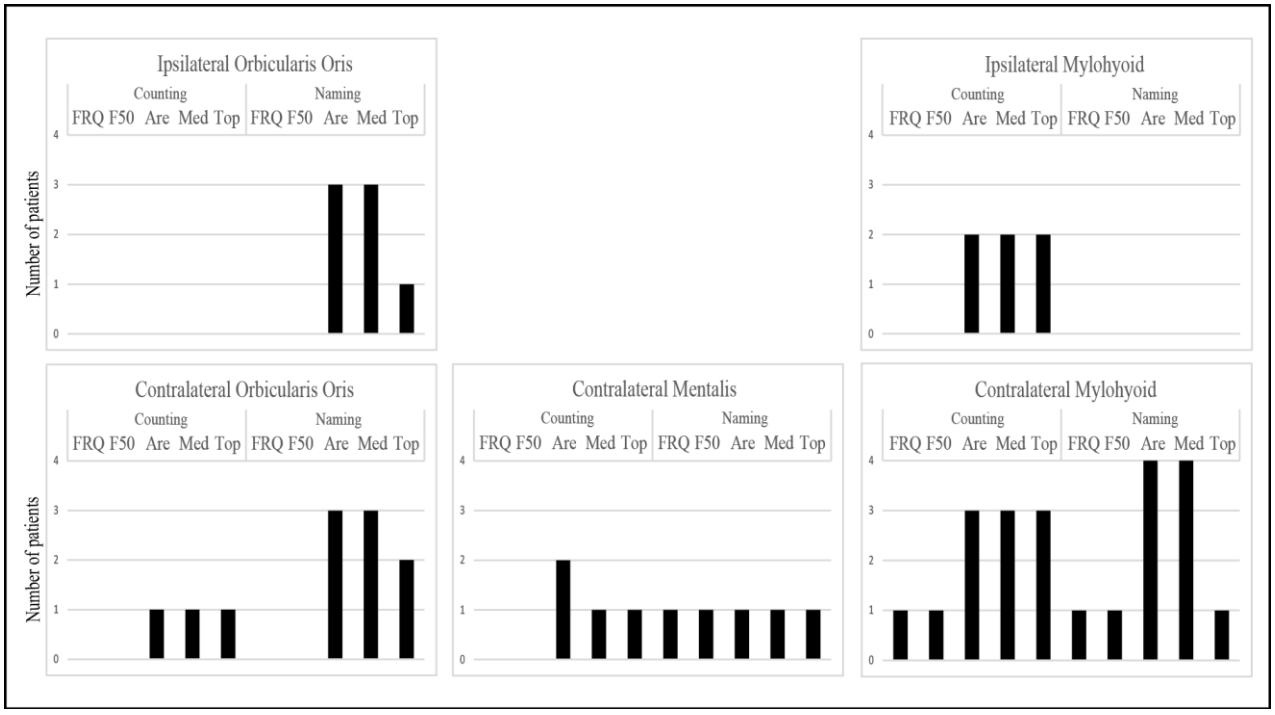

Figure 3.17 Statistical analysis on interferences obtained by stimulating M1 during speech tasks, in relation to natural performance. On the y-axis is the number of instances in which a statistically significant ($p<.05$) alteration of the values were induced by LF-DES, compared to natural performance. On the x-axis, the EMG-calculated parameters (FRQ = mean PS frequency, F50 = median PS frequency, Are= area under curve of PS, Med = mean RMS, Top = peak RMS) task-segregated are indicated. Notably, all parameters, statistically different, increased in relation to natural performance. No difference in ipsilateral *mentalis*.
PS: Power Spectrum, RMS: Root Mean Square

vPM and M1 program the articulatory activity independently from speech task

Since in the intraoperative setting the patients performed two speech tasks (counting and object picture naming), we investigated if the cortical areas involved in motor control of speech production play a different role in control of the two tasks. The cortical area included in this analysis was vPM,

while M1 was excluded because in no patients the LF-DES was applied on the same site during the performance of both tasks and Broca's area was excluded given the absence of an effect on phono-articulatory muscles in the task. To perform this investigation, each EMG parameter related to interferences in two speech tasks was compared among patients actually performing both tasks by means of: i) univariate (**Fig. 3.18** at left) analyses (GLM, see Materials and Methods section), independently from other factors; and ii) of an multivariate analysis (**Fig. 3.18** at right), considering the factor muscle (OO, MENT, MYLO) and body side (contra- and ipsilateral, not shown).

vPM

We compared the interference speech patterns induced by stimulation applied onto vPM during the two tasks. Only the stimulation during both tasks (object picture naming and counting) performed on the same site were considered (7 patients out of 45). In 2 patients the LF-DES induced an *Inhibitory/Recruiting* EMG pattern, in 3 patients a *Pure Inhibitory* pattern and in 2 patients a *Clonic-like* pattern**.** The univariate and multivariate analyses applied to each EMG calculated-parameters related to two speech tasks demonstrated that the LF-DES has the same effect onto vPM during both tests ($p>0.05$, **Fig. 3.18**). Data suggest that vPM exerts the same role in motor control of the phono-articulatory muscles independently from the language stream involved to form the word (object or number).

vPM and M1 program the activity of the contra- and ipsilateral muscles

Since the activity of phono-articulatory both contra- and ipsilateral muscles to the stimulated hemisphere was recorded, we evaluated the effect of vPM and M1 stimulation during task performance relative to the body side. To perform this investigation, we considered together interference-trials related to both speech tasks, since in the previous analysis we demonstrated that the LF-DES interference effects are superimposable in both tasks. Each EMG parameter related to interferences was compared with a univariate analyses for the factor "body side" (contra- and ipsilateral) (GLM, **Fig. 3.19**, **3.22**, see Materials and Methods section) and with a multivariate analysis (**Fig. 3.20**) including the factor "muscle" (OO, MENT, MYLO). In vPM the interference-effect induced by LF-DES was also assessed with a multivariate analysis including the factor "pattern of interference"(*Clonic-like, Inhibitory/Recruiting, Pure Inhibitory*).

vPM

In 31 patients was possible to apply the statistical analysis. The univariate analysis (**Fig. 3.19**) demonstrated that left vPM stimulation affects the muscle activity bilaterally, with the same EMG patterns on both body sides. However the effect, related to RMS mean and PS area parameters, is

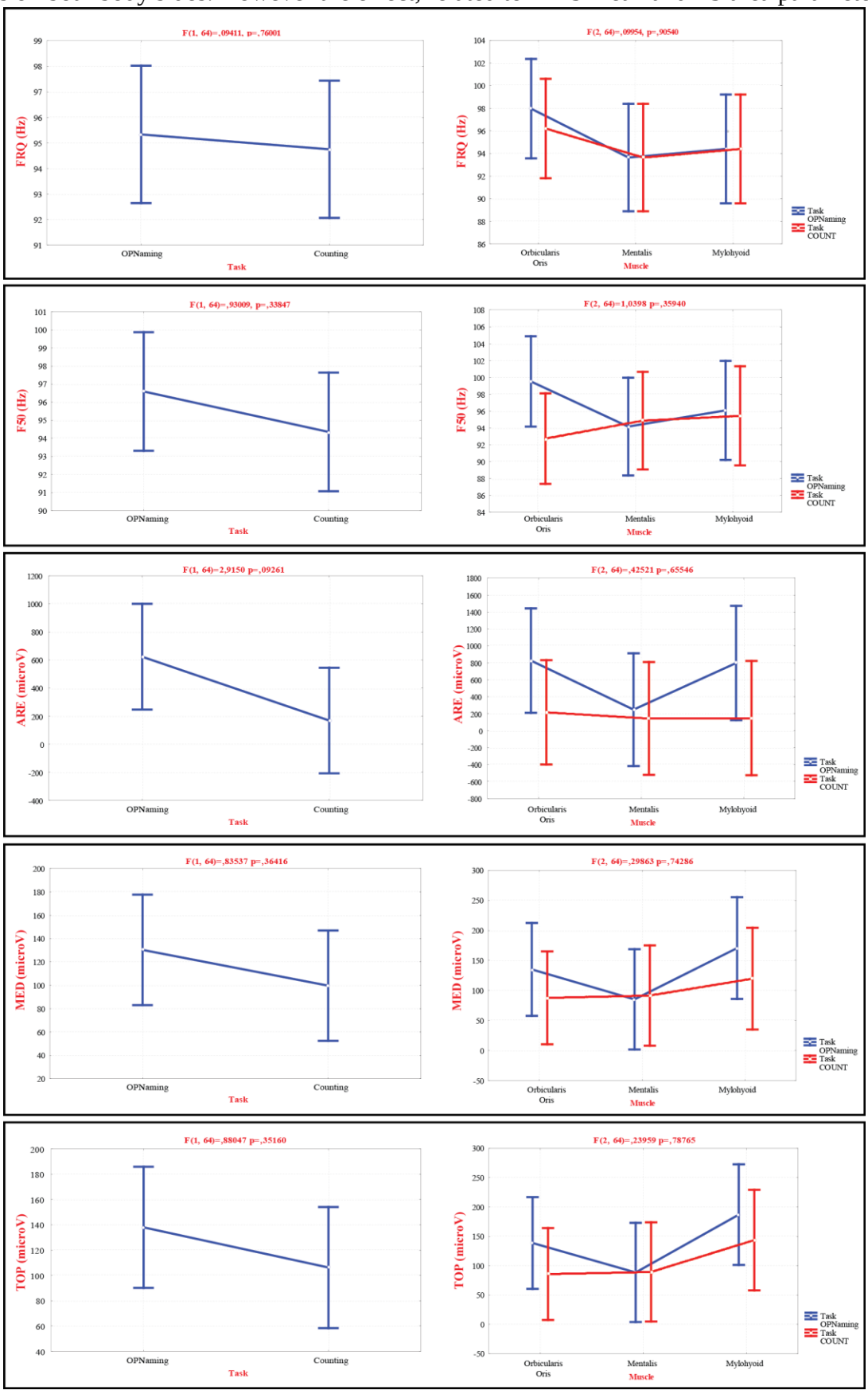

Figure 3.18 Population statistical analysis (N = 7, GLM see Materials and Methods section) on EMG traces related to interferences induced by LF-DES, when applied onto vPM, during counting AND object picture naming tasks to quantify if the induced interferences are statistically analogues in two tasks or if vPM controls majorly the production of one task in relation to other. In the first column (at left) are compared EMG parameters related to EMG interferes during OPNaming (object picture naming) in relation to Counting, independently from analyzed muscle and the body side (univariate analysis). In the second column (at right) are compared EMG parameters related to EMG interferes during OPNaming in relation to Counting, considering the factor muscle (multivariate analysis). The statistical results are reported on each graphs. From analyses appear that LF-DES, applied onto vPM, interferes with the phono-articulatory activity in analogous way during speech performances.
The vertical line indicates the confidence interval (95%). FRQ = mean frequency of power spectrum (PS); F50 = median frequency of PS; ARE = area of PS. MED = mean of root mean square (RMS); TOP = peak of RMS.

different and greater ($p<0.05$) in contralateral muscles. No statistical difference was observed ($p>0.05$) on frequency parameters (FRQ = mean frequency and F50 = median frequency). This result may suggest that the influence of vPM in motor units recruitment is more effective on the contralateral side However, the LF-DES has a more influence on ipsilateral *versus* contralateral muscles in frequency parameters (**Fig. 3.19**).

The multivariate analysis (**Fig. 3.20**) showed that the effect of vPM stimulation on contralateral side is significantly more correlated to the *Clonic-like* pattern with respect to the other EMG patterns. In order to disclose whether the "preferential" effect of left vPM simulation on the contralateral side is due only to its effect on the *Clonic-like* pattern, we evaluated the effect of vPM stimulation on ipsi and contralateral side by considering the *Pure Inhibitory* and *Inhibitory/Recruiting* patterns separately (**Fig. 3.21**) from the *Clonic-like* pattern. The univariate analysis (**Fig. 3.21** at left) confirmed the hypothesis showing that the vPM stimulation affects bilaterally muscles with no statistical difference in RMS and PS parameters when considering the two body sides in *Pure Inhibitory* and *Inhibitory/Recruiting* patterns. An additional multivariate analysis demonstrated that the LF-DES exerts the same effect on each recorded-muscle (not shown in figure). When considering the *Clonic-like* pattern separately, the univariate analysis (**Fig. 3.21** at right) demonstrates a clear greater effect of stimulation on the contralateral muscles, with the same effect on the different muscles recorded.

Conclusively, it could be suggested that vPM exerts a bilateral control (direct or indirect) on the activity of the phono-articulatory muscles. A greater effect is observed on contralateral muscles only when the stimulation induced the *Clonic-like* pattern.

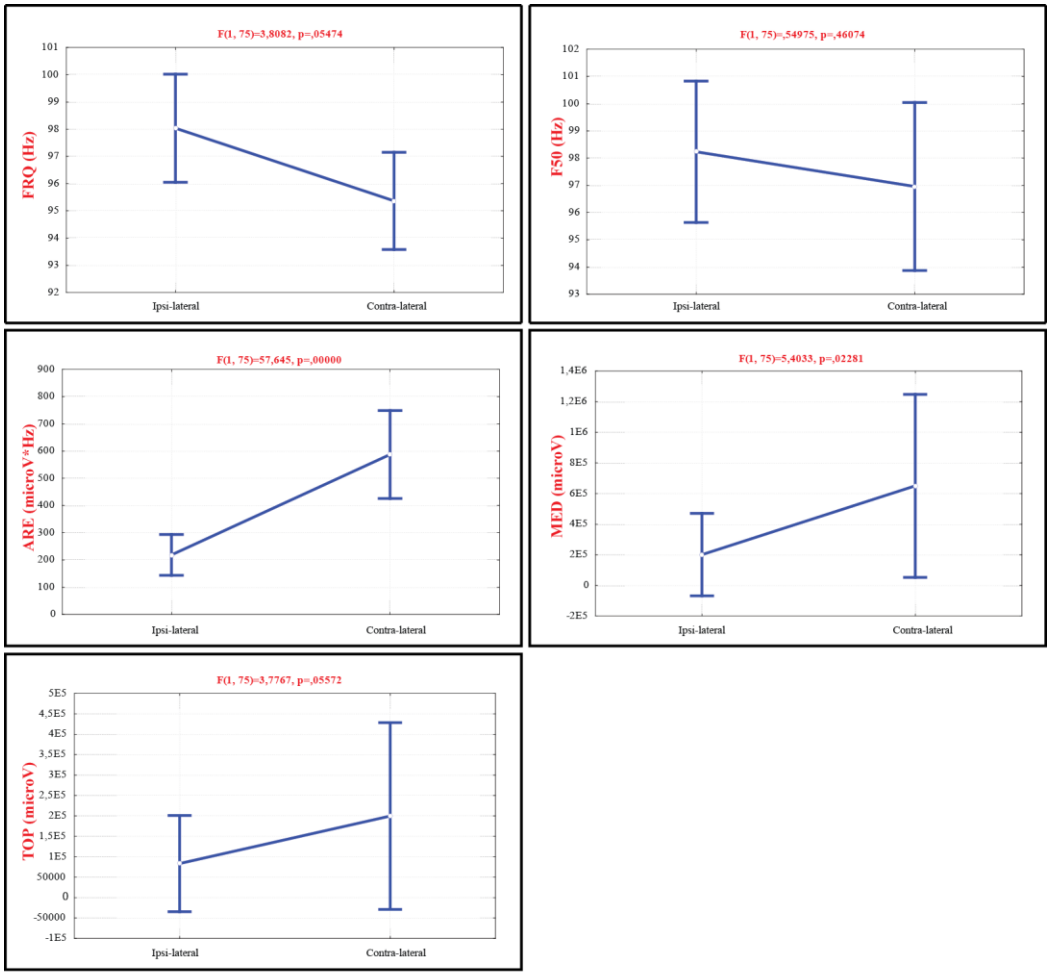

Figure 3.19 Population statistical analysis (N = 31, GLM see Materials and Methods section) on EMG traces related to interferences induced by LF-DES, when applied onto vPM, during both speech tasks to quantify if the LF-DES influences both contra- and ipsilateral muscles, independently from muscle and interference-effect. The statistical results are reported on each graphs. From analyses appear that LF-DES, applied onto vPM, interferes with the phono-articulatory activity of the contra- and ipsilateral muscles, with a major control on those contralaterals: PS area and RMS mean (med) are significantly different in two groups and RMS peak (top) has the same statistical inclination.
The vertical line indicates the confidence interval (95%). FRQ = mean frequency of power spectrum (PS); F50 = median frequency of PS; ARE = area of PS. MED = mean of root mean square (RMS); TOP = peak of RMS.

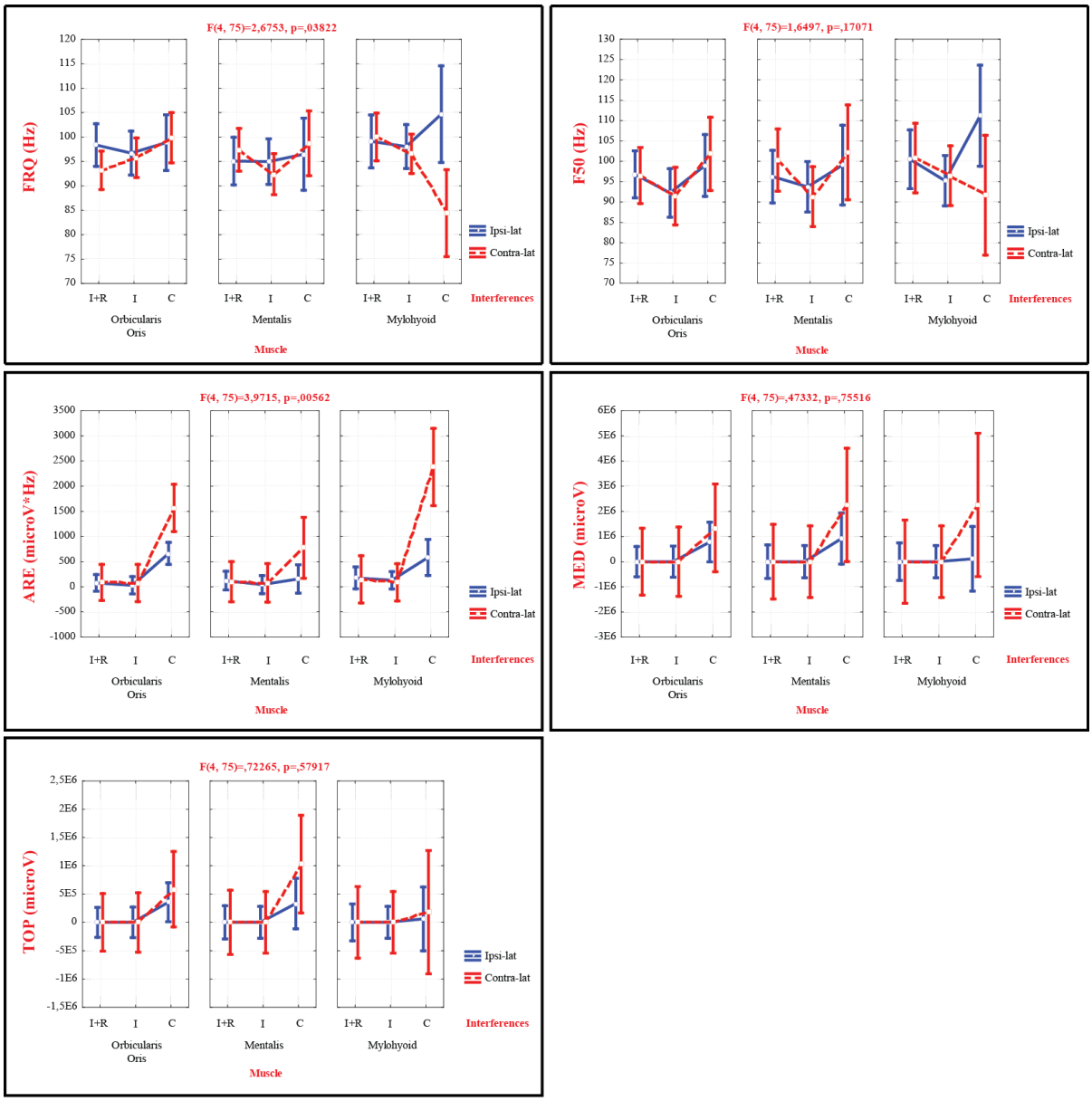

Figure 3.20 Population statistical analysis (N = 31, GLM see Materials and Methods section) on EMG traces related to interferences induced by LF-DES, when applied onto vPM, during both speech tasks to quantify if the LF-DES influences both contra- and ipsilateral muscles, considering the factor muscle and interference-effect. The statistical results are reported on each graphs. From analyses appear that LF-DES, applied onto vPM, interferes with the phono-articulatory activity of the contra- and ipsilateral muscles, with a major control on those contralaterals: PS area and RMS mean (med) are significantly different in two groups and RMS peak (top) has the same statistical inclination.
The vertical line indicates the confidence interval (95%). FRQ = mean frequency of power spectrum (PS); F50 = median frequency of PS; ARE = area of PS. MED = mean of root mean square (RMS); TOP = peak of RMS.

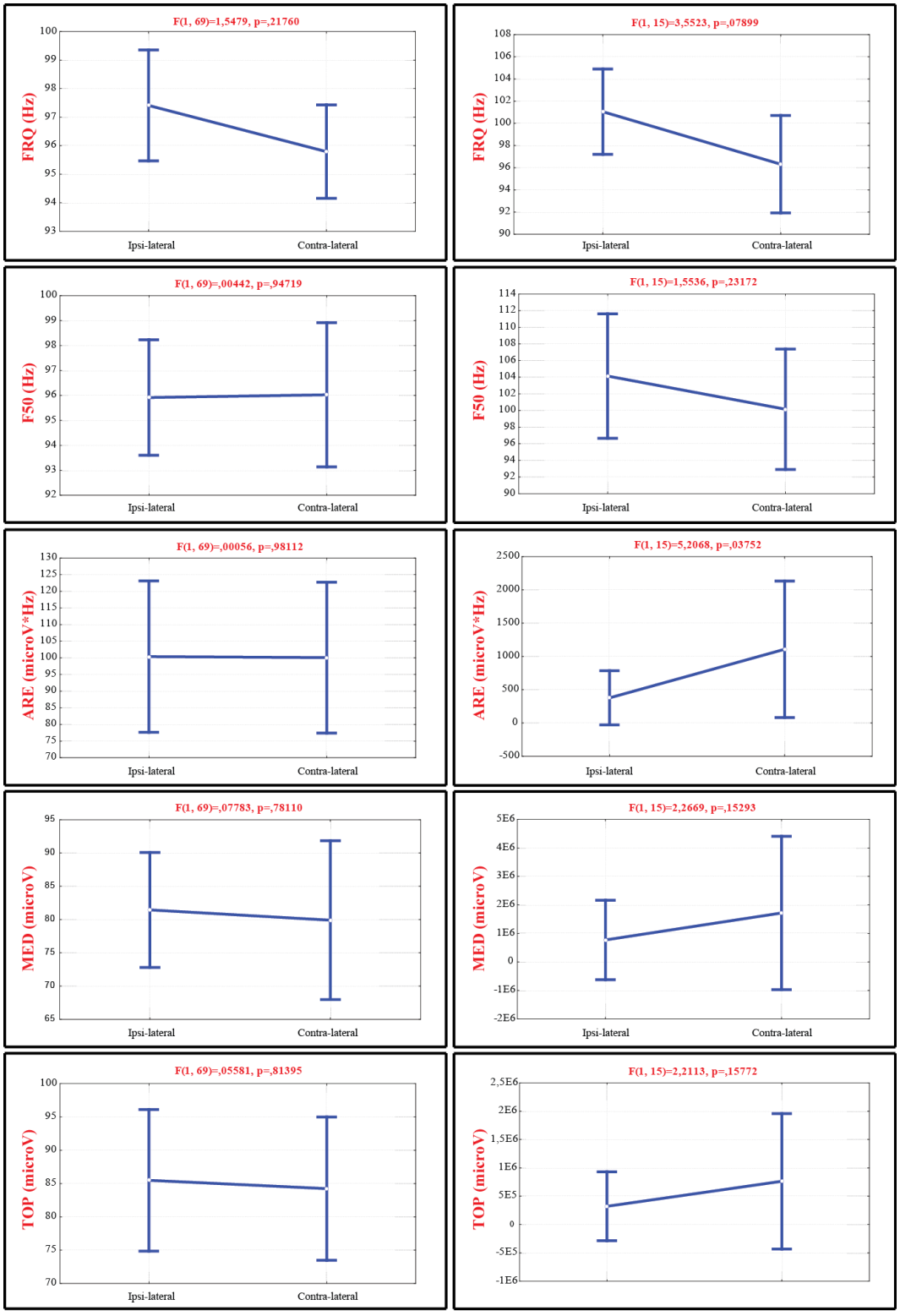

Figure 3.21 Population statistical analysis (N = 31, GLM see Materials and Methods section) on EMG traces related to Inhibitory and Inhibitory plus Recruitment patterns at left and to *Clonic-like* pattern at right to quantify as the LF-DES influences the contra- and ipsilateral muscles in EMG patterns. The statistical results are reported on each graphs. From analyses appear that in *Inhibitory* and *Inhibitory/Recruiting* patterns, the LF-DES influences the phono-articulatory activity of the contra- and ipsilateral muscles without statistical difference. While in Clonic-like pattern the LF-DES has a major influences on contralaterals muscles: PS area is significantly different in two muscle groups, and the peak (top) and the mean (med) of the RMS have the same statistical inclination.
The vertical line indicates the confidence interval (95%). FRQ = mean frequency of power spectrum (PS); F50 = median frequency of PS; ARE = area of PS. MED = mean of root mean square (RMS); TOP = peak of RMS.

M1

The univariate analysis (**Fig. 3.22**) for the factor "body side" (contra- and ipsilateral) demonstrated that M1 stimulation affects the activity oro-facial muscles bilaterally, although with a prevalent effect contralateral side.

Based on the results obtained in vPM for this analysis, the effect on EMG obtained during the counting and the object picture naming task were considered together. A multivariate analysis (not shown) was also performed to compare the effect of the LF-DES onto contra- and ipsilateral muscles,

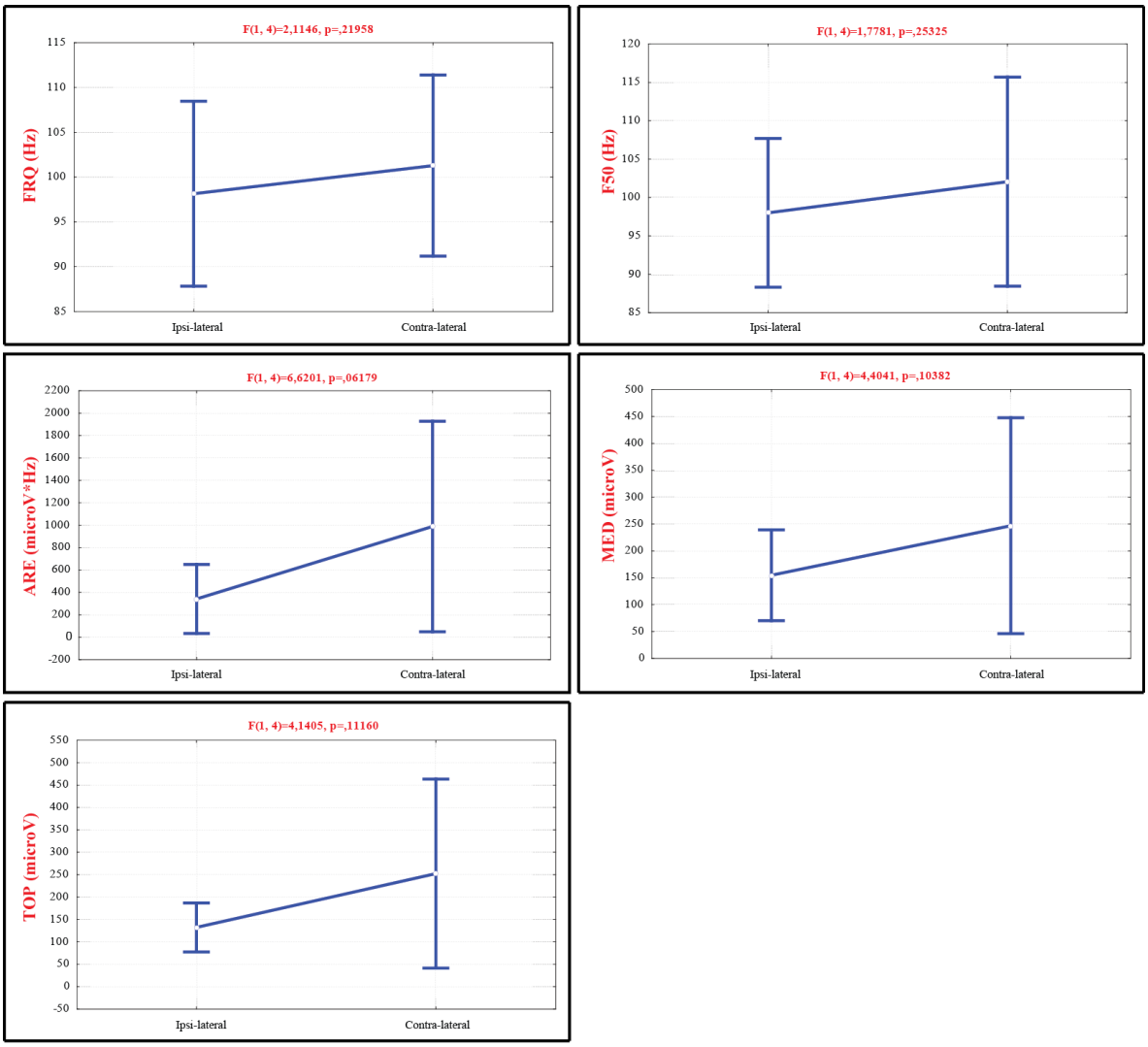

Figure 3.22 Population statistical analysis (N = 7, GLM see Materials and Methods section) on EMG traces related to interferences induced by LF-DES when applied onto M1, to investigate if M1 is involved in motor programming of the contra- and ipsilateral muscles and in what ratio. The statistical results are reported on each graphs. From analyses appear that M1 controls the phono-articulatory activity of both body side, without statistical prevalence. However, the analysis highlights a statistical tendency maid by M1 in motor control of the contralateral muscles *versus* ipsilateral. The vertical line indicates the confidence interval (95%). FRQ = mean frequency of power spectrum (PS); F50 = median frequency of PS; ARE = area of PS. MED = mean of root mean square (RMS); TOP = peak of RMS.

for the factor muscle (OO, MENT, MYLO). The analysis results support the highlights obtained in univariate analysis.

Thus, M1 effect is exerted bilaterally although with a greater effect on contralateral muscles. Interestingly this effect is similar to that obtained with vPM stimulation inducing the *Clonic-like* pattern

Cortical distribution of the stimulation sites inducing speech interferences

The stimulation sites related to speech-interferences were reported on the 3D reconstruction of the brain left hemisphere based on the MNI template (ICBM 152, see Materials and Methods section, **Fig. 3.23**). This reconstruction allowed to precisely localize: i) the position of the sites responsive to

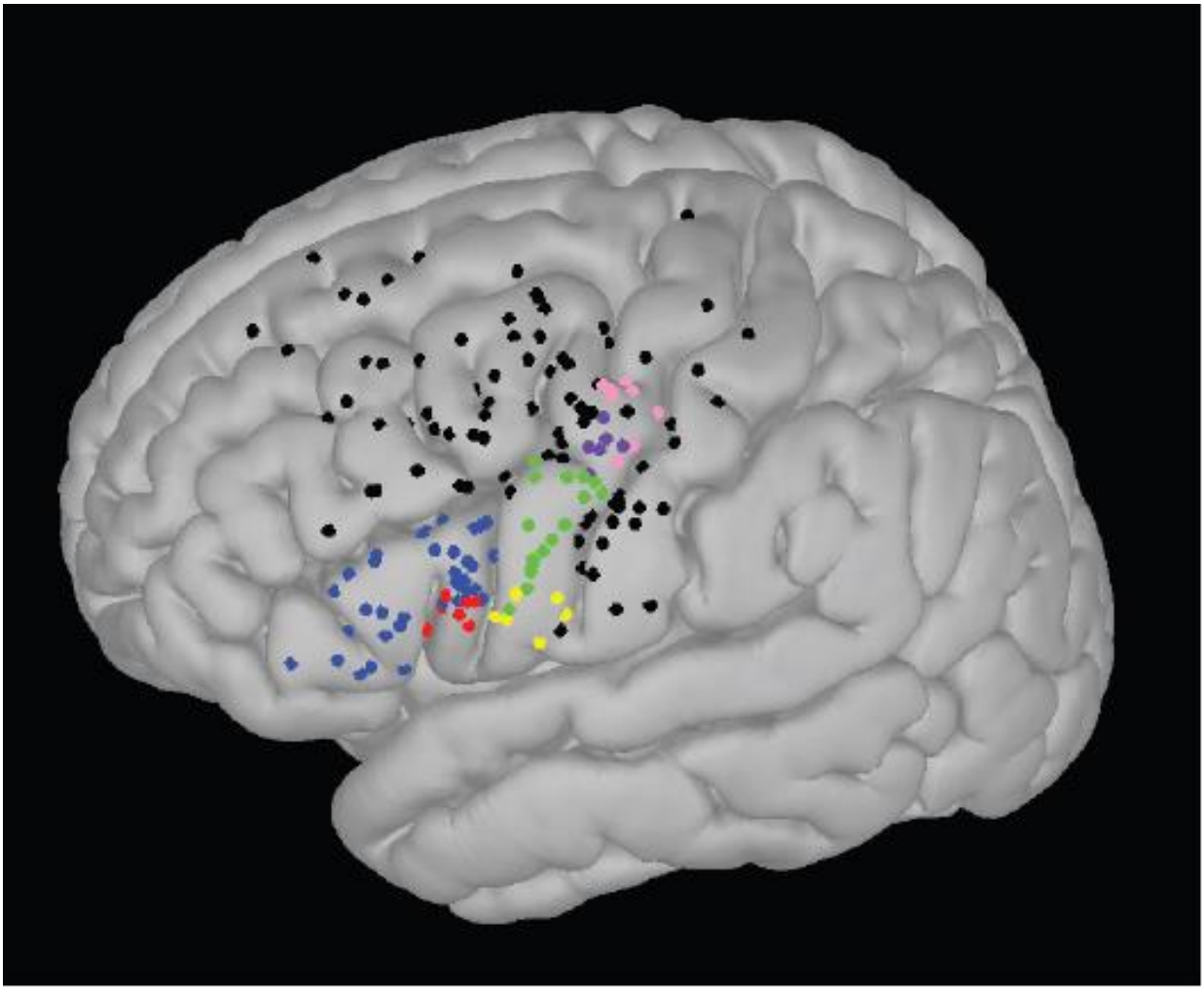

Fig. 3.23 3D map of stimulation sites in 30 patients out of 58. ICBM152 template. Blue dots over BA45-44 (Broca's area) represent the non-responsive sites to LF-DES (*ThreshI* and *SupraThreshI*) neither to motor level nor at phonological and semantic level; Red dots over vBA44 represent sites where *SupraThrehI*-LF-DES induces the *speech prevention* phenomenon; Yellow dots represent sites on vPM where LF-DES induces an EMG Pure Inhibitory pattern; Green dots represent sites on vPM where LF-DES induces an EMG Inhibitory/Recruiting pattern; Purple dots represent sites at functional border between vPM and M1 where LF-DES induces an EMG *Clonic-like* pattern; Pink dots represent sites on M1 where LF-DES induces speech arrest; Black dots represent sites where LF-DES has not effect on speech tasks.

the stimulation on Broca's area, vPM and M1; ii) the position of the sites not responsive to stimulation on the three areas; ii) the somatotopic organization of responsive sites and iv) the distribution of sites responsible to stimulation during speech task *vs* sites responsive to stimulation with MEPs in resting state condition (**Fig. 3.4 A**). Only the sites responsive to least three stimulations were reported on the map.

Broca's area (BA44-45)

Sites responsive to *SupraThreshI*-LF-DES during object picture naming/counting tasks clustered on ventral portion of the BA44 (vBA44, red dots in **Fig. 3.23**). In these sites, the *SupraThreshI*-LF-DES, applied before of the word/count articulation, induced the phenomenon of prevention of the motor output of the speech ("speech prevention"). This result was in agreement with the recent literature (Papoutsi et al., 2009; Hickok and Poeppel 2000; 2004; 2007) suggesting the vBA44 is involved in a preparatory phase to the articulatory program (phonetic encoding) of the words. *SupraThreshI*-LF-DES delivered on the same sites during ongoing word articulaiton failed to induce an effect, coherent with a role played by vBA44 in phonetic encoding of the words and not in direct motor control of speech. Interesting is the representation of blue dots (in **Fig. 3.23**) that were not responsive to stimulation (both *ThreshI*-LF-DES and *SupraThreshI*-LF-DES) for semantic and phonological paraphrase, in contrast with the most recent literature reporting (Tate et al., 2014, 2015) the occurrence of semantic and phonological disturbances upon stimulation of Broca's area.

vPM

Given that it was possible to reconstruct the 3D MRIs only in patients with a tumour not displacing the cortex, the sites represented (**Fig. 3.23**) on the map related to vPM stimulation belonged to 25 out of 45 patients. The 3D reconstruction cleary shows that the different EMG patterns of interference clustered in different positions on the vPM:

- *Group 1*: sites (N = 6; purple dots) inducing the *Clonic-like pattern* clustered on the dorsal portion of the pre-central *gyrus*, proximal to the functional border between M1 and vPM;
- *Group 2*: sites (N = 6; yellow dots) inducing the *Pure Inhibitory pattern* clustered in the most ventral portion of the vPM;
- *Group 3*: sites (N = 17; green dots) inducing the *Inhibitory/Recruiting pattern* clustered between the sites related to *Pure Inhibitory* pattern and the *Clonic-like* pattern.

In **Figure 3.24** the probabilistic somatotopical map (lateral view of the left reconstructed hemisphere, and dominant for language in 25 reconstructed brains) obtained by means of MNI coordinates of each stimulation site is also represented. The density estimation for each site was reported with a color

code indicating the probability level of site density obtained by interpolating MNI Y and Z values. Figure shows three activity peaks, strictly corresponded to three interference EMG patterns. The 2D coordinate (y, z) of the density peak related to *Clonic-like* pattern was -2.8, 45, for the *Inhibitory/Recruiting* pattern was 4.9, 24.7 and for the *Pure Inhibitory* pattern was 6.6, 15.5.
This organization, along the ventral portion of vPM, reflected a behavioral/functional somatotopy of neurons involved in motor programming of the phono-articulatory muscles and not a muscle somatotopy, as described by Bouchard and colleagues (2013).

M1

The sites (N=8) related to M1 stimulation clustered in the most ventral and lateral part of M1, at the border with the portion of vPM inducing the interference *Clonic-like* pattern. When comparing the area of "phono-articulatory interference" on M1 *vs* vPM, the latter shows a wide area which, based on the interference effect, seems to be involved in phono-articulation.

Non-responsive sites

The sites (N = 84) where the stimulation failed to induce motor or semantic linguistic impairments, therefore the sites were called "non-responsive sites". These points clustered over dorsal portion of the frontal lobe and over anterior parietal lobe when exposed regions by surgical flaps.

HARDI reconstruction of the fibers extremity reaching Broca's area

The main white matter bundles of the language network were reconstructed in six patients (out of 58) and the cortical terminations of tractography analysis plotted in the MNI space (**Fig. 3.25**). All the six (out of 20) patients were located on Broca's area during speech tasks and the "speech prevention" effect was obtained with *SupraThreshI*-LF-DES. The tractographic analysis highlights the terminations of the arcuate *fasciculus* (AF), the superior longitudinal *fasciculus* component II and III (SLF II-III) and of the frontal aslant tract (FAT) emerging in both vPM and in the ventral portion of BA44 (**Fig. 3.25 B** in green, pink and blue respectively) where the sites related to "speech prevention" phenomenon clustered (red dots in **Fig. 3.25 B**). Parallel to their termination in vBA44 the same systems of fibers emerge also in the most ventral portion of vPM, where the improper articulation of speech production was observed (**Fig. 3.25**).

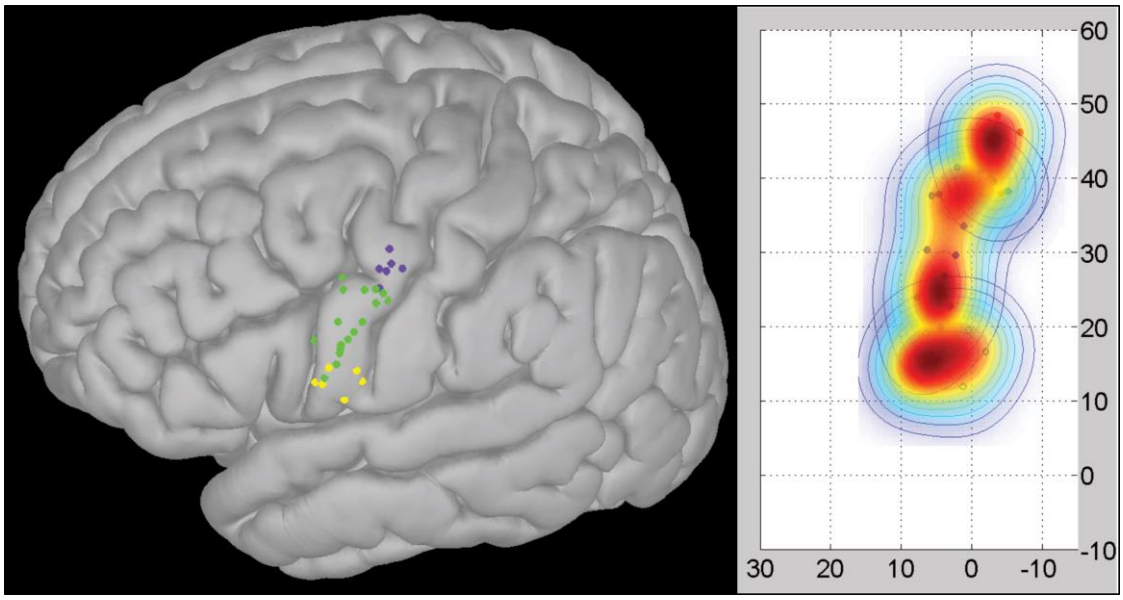

Figure 3.24 The left panel shows the localization of three different EMG clusters, obtained when DES was applied on vPM during speech tasks. In yellow are represented the stimulation sites on which DES induced a *Pure Inhibiotry* pattern; in green are represented the stimulation points related to *Inhibitory/Recruiting* pattern; in purple are represented the stimulation sites related to *Clonic-like* pattern. The right panel represents the probabilistic somatotopical map (lateral view of the left reconstructed hemisphere) obtained by means of MNI coordinates of each stimulation site. The density estimation for each site was reported with a color code indicating the probability level (higher in red, lower in light blue) of site density obtained by interpolating MNI Y (x axis) and Z (y axis) values.

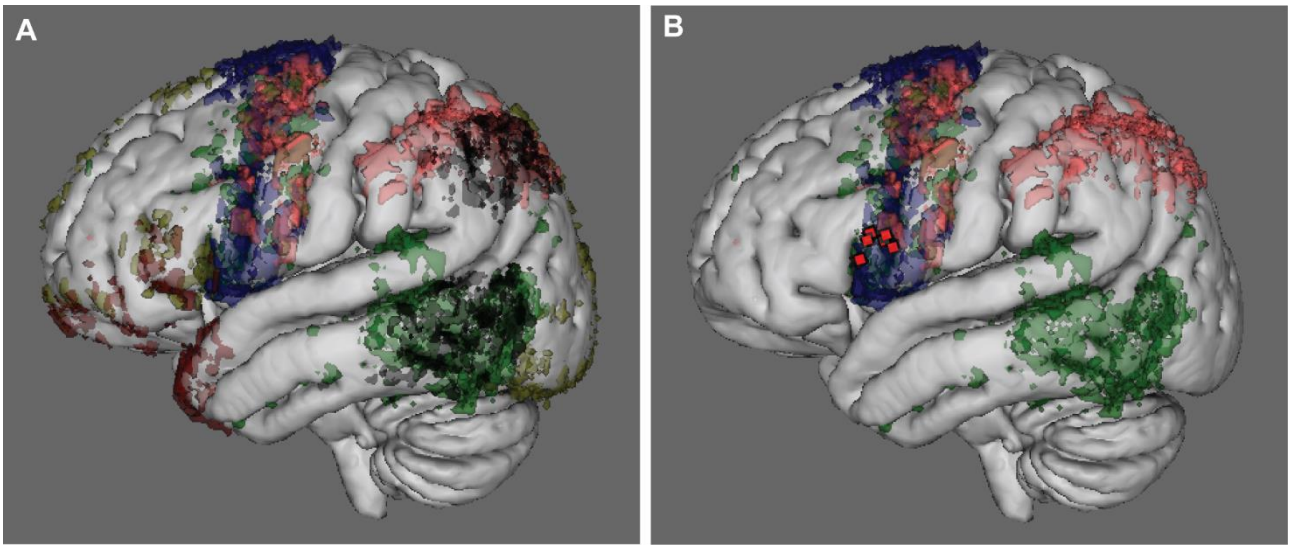

Figure 3.25 The panel **A** shows the cortical terminations, in voxels, that were reached by the language fibers principal tracking algorithm in at least two patients: arcuate *fasciculus* (AF, in green), the superior longitudinal *fasciculus* component II and III (SLF II-III, in pink), the superior longitudinal *fasciculus* component temporal-parietal (SLF-tp, in black), the frontal aslant tract (FAT, in blue), the inferior frontal-occipital *fasciculus* (IFOF, in yellow) and the uncinate *fasciculus* (UF, in red). The terminations of the arcuate *fasciculus* (AF), of the superior longitudinal *fasciculus* component II and III(SLF II-III) and of the frontal aslant tract (FAT) are shown in panel **B**: in the inferior frontal *gyrus*, these tracts reach the ventral portion of BA44 where the stimulation sites related to "speech prevention" phenomenon (red dots) were found.

3.2 COMPARISON BETWEEN THE 3D MOTOR and SPEECH-RELATED MAPS

Conclusively, in order to investigate whether the sites positive for a motor output overlapped with those positive for speech-interference, the comparison of two 3D maps reconstructed from stimulation points in the two different conditions (rest state and speech tasks) was performed (**Fig. 3.26).**

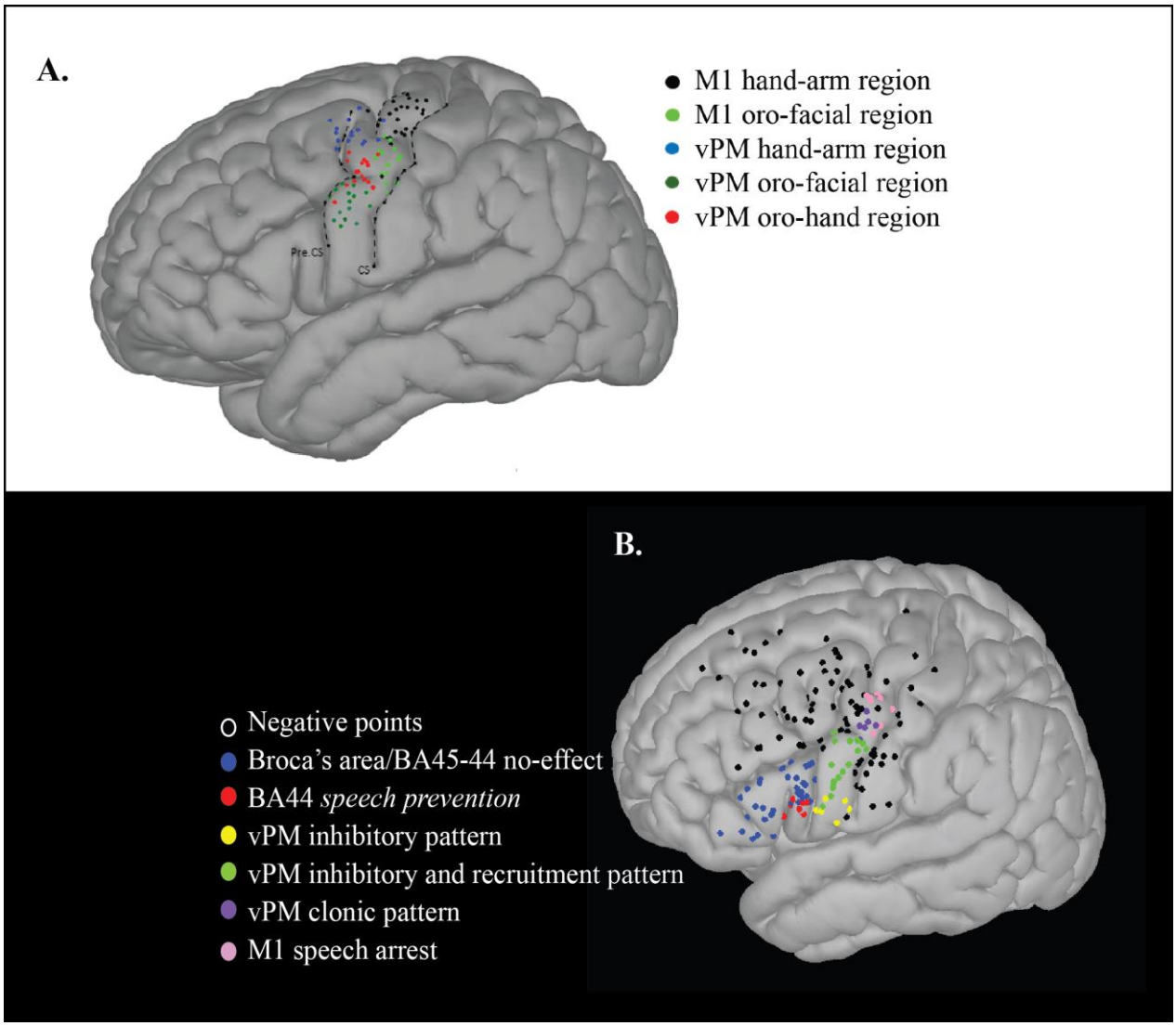

Figure 3.26 3D maps related to left stimulated hemisphere in all 70 patients (dominant hemisphere for language in all patients). In panel **A** are reported the stimulation sites from that MEPs were evoked by HF-DES in resting state condition. In panel **B** are reported the stimulation sites related to the motor-interferences and the negative points obtained by LF-DES during speech tasks.

See Figure 3.4 and 3.20 for details.

By merging the two maps, it emerged that:

Broca's area does not show motor properties. No MEP was evoked by HF-DES in resting state condition, neither in oro-facial nor hand-arm muscle district (Panel A). These data were confirmed by LF-DES applied on the same area in pre-activated condition (stimulation points not shown, EMG recording in **Fig. 3.1**). From Panel B, it emerges a lack of evidence of motor, semantic and phonological properties related to Broca's area, more reasonably exerting a role in phonetic encoding of the single words ("speech prevention", red dots).

vPM shows a "gesture somatotopy" rather than a muscle somatotopy with the most ventral region devoted to speech gesture in absence of motor output. Panel *A:* in more ventral sector of vPM no MEPs were obtained, while more dorsally a proper motor output was elicited form: i) oro-facial muscles (dark green dots); ii) oro-hand muscles (the "transition zone", red dots); iii) hand-arm muscles (blue dots). Panel *B*: the most ventral sector of vPM, where no MEPs in resting state condition were obtained, hosted the stimulation sites positive for the speech interference characterized by the *Pure Inhibitory* pattern (yellow dots). The *Inhibitory/Recruiting* patterns (green dots) matches the area hosting the oro-facial motor responses, while the *Clonic-like* pattern (purple dots) clustered proximal to the M1 responses.

By merging the motor and the speech-related maps, it could be suggested that the most ventral sector of vPM is dedicated almost exclusively to motor control of phono-articulation, which could be indirectly exerted through an inhibitory effect, thus the absence of motor responses. While the ventrolateral vPM seems to be involved in selection of motor actions involving the oro-facial, including the phono-articulatory function, and hand-arm muscles. Supporting this view is the observation that vPM seems characterized by a behavioural somatotopy (and not muscle somatotopy): in its more ventral portion the HF-DES does not evoke MEPs in resting state condition, but the LF-DES application during speech tasks induces a motor inhibition of the phono-articulatory muscles; where the HF-DES evokes oro-facial MEPs, the application of the LF-DES during speech tasks induces a motor simultaneous inhibition and recruitment of the phono-articulatory muscles, a very different effect when compared to the effect obtained during the same tasks when stimulating M1 or the vPM close to M1 border showing a prevalent excitation of the muscles represented in the stimulated area. Notably the LF stimulation, during speech tasks, in the transition zone (oro-hand) and in hand-arm vPM representation, the phono-articulatory function is not disrupted, suggesting that this portion of vPM controls other movements of oro-hand districts.

The oro-facial M1 representation is a small cortical region. The oro-facial M1 representations of both panels are superimposed, suggesting that in this region are located neurons controlling oro-facial muscles, independently from goals of the actions. This area appears small,

probably, because the remaining part of oro-facial M1 maybe located within the bank of the central sulcus not reached by DES.

4. *Discussion*

The language is a unique and essential instrument of human thought and communication. Thanks to this ability humans can produce and understand a boundless number of expressions, comprehensible to others sharing similar knowledge. It is suggested that language is based on a computational mechanism, realized by a complex neural network that produces an array of structured expressions. This array depends on the interaction of the initial genetic endowment, general principles, external data and external laws of form. The genetic factor in turn is composed of the so-called "universal grammar" that together to capacity to form a context-free language distinguish the human language from others mammalian language.

The human language, as we know it, is composed of three components: the mental expressions, the sensory-motor interface and the internal conceptual-intentional interface (Berwick et al., 2013). Aim of the PhD project was to investigate three cortical areas in the frontal lobe involved in the sensory-motor interface. The language network is composed of a complex cortical and subcortical structures generating and controlling the precise sequence of motor events involved in the production of sounds, at a rate up to six to nine syllables per second, or 20 to 30 phonetic segments per second (Kent, 2000), occurring once the linguistic form of a word or a phrase has been elaborated. To operate such an intricate function, the system finely coordinates the phono-articulatory apparatus (Fink, 1986; Berwick et al., 2013) this is composed of about 100 muscles (Kent, 2000), by means of corticobulbar tract, originating from M1/BA4, in turn controlling the cranial nerves innervating oro-facial muscles. This project focused on three cortical frontal areas: the primary motor cortex (M1/BA4), the ventral pre-motor cortex (vPM/vBA6), the Broca's area (BA44-45).

The study was conducted in 70 patients affected by gliomas affecting the left hemisphere (linguistic dominance for all patients), requiring the exposure of the areas of interest. The surgical removal performed with the aid of the *brain mapping* technique while recording of the electrical activity of some phono-articulatory muscles (EMG) involved in speech production, the electrical stimulation of M1, vPM and Broca's was allowed at rest and during speech tasks. According to the literature, all these areas are supposed to be motor areas and therefore to control directly or indirectly, by modulating the motor program, the activity of oro-facial -including the phono-articulatory- and hand-arm muscles. The motor properties of the areas were assessed by recording the EMG activity of muscles during stimulation in two different conditions: 1) in a resting and in a pre-activated state, to

investigate the occurrence of motor responses in the oro-facial and hand-arm muscles in each cortical area; 2) during speech tasks (object picture naming and counting tasks), investigating the effect of stimulation on the three areas on task performance. As a novelty, with respect to the available neurosurgical literature during the stimulation, the electrical activity of some muscles involved in phono-articulation was recorded thus allowing the offline quantitative analysis of the muscle activity. With this analysis, the pattern of motor unit recruited during the performance in absence of stimulation -*natural performance*- was compared with the pattern recorded during the intraoperative speech tasks when the stimulation was applied onto three cortical areas -*LF-DES interference*-. The analysis of the distinguishing alterations in the pattern of motor unit recruitment, specifically associated to the stimulation of the three different cortical areas, allowed to add new evidence in the debate on the role these three areas in motor control of phono-articulatory production during speech.

4.1 COMPARISON OF MOTOR RESPONSES IN M1, vPM AND BROCA'S AREA

The analysis of motor response obtained by stimulating M1, vPM and Broca's area allowed to localize the oro-facial and hand-arm districts in all the areas. DES was applied when oro-facial and hand-arm muscles were at rest and then in a pre-contracted state. The DES paradigms used were two: LF (Low Frequency) and HF (High Frequency).

LF-DES on M1/BA4 always caused a fast muscular recruitment, irrespectively from the excitability muscle state (**Fig. 3.1**). This effect was expected, since M1 is directly connected with motoneurons controlling oro-facial and hand-arm muscles by corticobulbar and corticospinal tracts. Differently, LF-DES on vPM/vBA6 caused both excitatory and inhibitory effects on distal muscles, the former effect rarely observed in resting or slightly tonically pre-contracted muscles and, when it was the case, it showed a very slower recruitment compared to M1 responses. This behaviour of vPM responses could be explained by its dense cortico-cortical connections with M1 *(*Kujirai et al., 1993; Tokuno et al., 1997; Kobayashi et al., 2001; Paradiso et al., 2005; Simonyan and Jürgens, 2005; Pilurzi et al., 2013) and not direct connections with motoneurons. This different connections type explains the slower muscular recruitment when vPM was stimulated. While the inhibitory effect on tonic muscle pre-activation might recall the "stimulus-induced negative responses" evoked by stimulation of the negative motor areas on human brain (Luders et al., 1995). These areas, when stimulated, produced an inability to perform voluntary movements or to sustain an ongoing muscle contractions, as in our case. Therefore, when stimulating M1 and vPM with LF-DES, it is possible to obtain a motor response in both muscle districts (oro-facial and hand-arm) and in both muscle conditions (rest and pre-contraction). However, the motor responses elicited by vPM were lower and slower compared to M1 responses, suggesting that vPM is a less excitable area, probably requiring multi-synaptic summation to trigger a response (Kujirai et al., 1993; Tokuno et al., 1997; Kobayashi et al., 2001; Paradiso et al., 2005; Simonyan and Jürgens, 2005; Pilurzi et al., 2013). Notably, when LF-DES was applied on Broca's area consistently failed to show any motor output, irrespective of the muscle district (oro-facial and hand-arm) and of the excitability muscular state (rest or pre-contraction), suggesting that probably Broca's area has not motor properties or, alternatively, that LF is not the adequate protocol to stimulate this area.

Animal studies suggest that the high frequency stimulation (by repetitive intracortical microstimulation) is the most effective paradigm to elicit responses from primary (M1/BA4) and not-

primary motor cortices (Porter and Lemon, 1993). In our patients, HF-DES on M1/BA4 and vPM/vBA6 evoked positive motor responses in oro-facial and hand-arm muscles, and failed, again, to induce any motor responses when it was applied on Broca's area. The lack of motor responses to Broca's area stimulation with LF-DES and HF-DES reasonably challenges the inclusion of Broca's area in proper motor areas. However, for many years, Broca's area was considered the human region homologous to monkey's F5. We remember that monkey's F5 is the cortical localization site of mirror and canonical neurons and, when stimulated with intracortical microstimulation (ICMS) induces a muscular activation (Cerri et al., 2003; Kraskov et al., 2009). Should Broca's area be the homologous area to monkey's F5, it would be expected to induce motor responses to stimulation. Based on our results, Broca's area cannot to be considered the human homologous area to monkey's F5 and, consequently, cannot be considered the human homologous area to monkey's F5 hosting mirror neurons, challenging the F5 evolutionary root of human language and the mirror "motor theory of speech perception" (Di Pellegrino et al., 1992). This criticism is reinforced by neuroimaging studies showing that the sector of Broca's area activated in the actions observation (*pars triangularis* or BA45, Grafton et al., 1996) was not also activated in the actions execution (*pars opercularis* or BA44, which is actually the most relevant functional feature of the mirror neurons, neither in the actions imagination (Grafton et al., 1996). In fact, the cortical area belonging to both the mirror neuron system and to the language production system was identified (Cabinio et al., 2010; Cerri et al., 2015) based on activation during action execution, action observation and phonological fluency. From these studies, it emerged that vPM fully and consistently satisfies the requirements, while Broca's area does not. Moreover, lesion studies demonstrated that patients with lesions of Broca's area show deficits in speech production and sometimes in action comprehension, but never in perception of language.

In non-human primates the somatotopic organization of M1 and of vPM has been revealed by anatomical and electrophysiological investigations (Dum and Strick, 2005, Boudrias et al., 2010a,b). These studies provided a fundamental reference for investigation of M1 and vPM in human, however a clear description of the anatomy-functional subdivision of M1 and vPM is lacking. Principally, it is reasonable to hypothesize that the human vPM may be different compared to primate's vPM due to the increased complexity of the human sensory-motor system as, for example, the phono-articulatory component. ICMS, retrograde labelling and single unit recording from macaque brain have shown that motor outputs (MEPs) from a single muscle are represented multiple times over a wide region of the motor cortices (Andersen et al., 1975; Porter and Lemon, 1993; Rathelot and Strick, 2006, 2009; Boudrias et al., 2010a,b) and human fMRI studies have confirmed this multiple, overlapping structure of motor outputs (Sanes et al., 1995; Schieber, 2001). Based on this organisation, it has been

suggested that when a complex movement has to be performed, the horizontal connections within the motor cortex link ensembles of neurons that coordinate the ultimate pattern of discharge of the spinal motoneurons driving the different muscles needed to perform the planned movement (Lemon, 1988; Porter and Lemon, 1993; Barinaga, 1995; Schieber, 2001). In our study, the motor responses (MEPs) elicited in the same sample of muscles (two oro-facial muscles and four hand-arm muscles) were recorded when mapping M1 and vPM under the same conditions –at rest- were compared. The results show that single positive sites – cortical sites that, when stimulated, activated a single muscle among those recorded – were more common in M1 than in vPM (53% *vs* 38%). The more focused muscle representation in M1 is consistent with the idea of a surround inhibition in the fields around the stimulated spot, mediated through GABAergic transmission, potentially important for selective execution of desired movements (Mink, 1996; Ziemann et al., 1996; Sohn and Hallett, 2004). For example: voluntary execution of movements, as well those involving a single digit, are rarely isolated contractions of one muscle, but require simultaneous control of many different hand and forearm muscles acting on different fingers and joints (Schieber, 1991). In this case, the pyramidal neurons in M1 exert excitatory influences on their post-synaptic targets via their intra-cortical axon collaterals synapsing on other pyramidal neurons, as well as on cortical inhibitory interneurons (Hendry and Jones, 1981; Markram et al., 1998; Silberberg and Markram, 2007). The connections between pyramidal neurons provide feed forward excitatory interactions between groups of cells related to the same movement, whereas the connections with inhibitory interneurons may form a basis for surround inhibition between cortical zones related to the activation of different muscles (Keller and Asanuma, 1993). When we compare M1 with vPM, for example for hand-arm district, it may be that in M1 the distribution of excitability -due to the peculiar architecture of surrounding inhibition- is such that the stimulation of a complex and overlapping muscle representation can still emerge as a single muscle response, reflecting the net strength of outputs to that particular muscle when compared to those of other neighbouring muscles (some of which were not monitored in our study). The excitability in vPM, instead, may be distributed in a different fashion, allowing the emergence of similarly weighted outputs to multiple muscles.

Moreover, in M1 the number of single positive sites was higher in the hand-arm group than in the oro-facial group (70% hand-arm *vs* 30% oro-facial), while in vPM an equal percentage of such sites was found in both hand-arm and oro-facial groups (**Fig. 3.3**). The different proportion of single upper limb *vs* cranial muscle response found in M1 could reflect a particular role of the human M1 in controlling hand-arm movements in relation to those oro-facials. However, since M1 is mainly located within the bank of the central sulcus, particularly the more caudal 'new' M1 (Geyer et al., 1996; Rathelot and Strick, 2009), our results could reflect the particular difficulty of activating the

oro-facial region of M1 with a stimulating probe on the cortical surface. It may also be that oro-facial and hand-arm neurons are differentially sensitive to DES of the cortical surface. This different somatotopic localization –bank *vs* surface- of oro-facial and hand-arm representations reflects the difficulty in investigation of M1 in speech production and as a consequence the inferior number of stimulations on it. In contrast, in vPM both the oro-facial and the hand-arm representations are located on the convexity of the pre-central *gyrus*, and therefore both were more accessible to DES.

When we consider vPM, the number of single positive sites of oro-facial group was equal to that of the hand-arm group. Regarding hand-arm muscles, the sampled-muscles most often were *extensor digitorum communis* (EDC) and *abductor pollicis brevis* (APB), followed by *first dorsal interosseous* (FDI), consistent with the role in grasp of the ventral pre-motor area (F5) in monkeys (Raos et al., 2006; Umiltà et al., 2007). During hand grasp, EDC and APB muscles are responsible for pre-shaping and opening of the hand, while FDI muscle is fundamental in characterizing the type of grip, in particular the precision grip (Brochier et al., 2004). In humans, neuroimaging data show a significant engagement of motor areas, and in particular, the ventral sector of the pre-motor cortex (vPM), in highly skilled movement such as precision grip and haptic manipulation (Binkofski et al., 1999; Ehrsson et al., 2001). This feature could be reflected in the dominant representation in vPM of neurons controlling coordination of muscles such as EDC, APB and FDI for precision grasp. In contrast, a less dexterous grasp, such as a whole hand grasp or power grip that involves all hand muscles used in a less fractionated pattern, is known to be a less effective in fMRI-based activation of the human vPM (Ehrsson et al. 2000; Begliomini et al. 2007). Interestingly, one of the muscles contributing to this grasp, the *abductor digiti minimi* (ADM), was not found among vPM single positive sites, but only among multiple positive sites.

During intraoperative mapping, in fact, many multiple positive sites were disclosed (in M1 and in vPM). When stimulated, these sites elicited two types of responses: i) simple muscle combination responses (MPs-SMc) – activating several muscles within same effector: oro-facial or hand-arm– and ii) complex muscle combination response (MPs-CMc) – activating several muscles from both, mouth and hand, effectors (*oro-hand responses*). Such multiple responses were more prevalent in the vPM than in M1 (62% *vs* 47%), suggesting a more complex motor organization of this area with respect to M1. The multiple positive sites found in M1 evoked co-contraction of multiple muscles within the same effector (*orbicularis oris* (OO) and *mylohyoid* (MYLO), EDC and ADM, FDI and APB or all hand muscles), suggesting that oro-facial and hand-arm representations are somatotopicaly separated in M1, the oro-facial area in ventral-bank and the hand-arm area on the surface. While the multiple positive sites in vPM elicited mostly *oro-hand responses*, i.e. co-activation of three or all oro-facial and hand-arm muscles. These muscle combinations are almost exclusive features of the vPM (35%

in vPM *vs* 4% in M1), emerging as a distinctive group clearly segregated between a dorsal hand-arm representation and a ventral oro-facial representation, as indicated by the 3D reconstruction (**Fig. 3.4**). In 3D somatotopic map appears also a more ventral portion of vPM from that nothing MEPs is obtained, neither in oro-facial, hand-arm nor foot-leg muscles.

Analysis of MEPs latency: M1 *vs* vPM

The first analysis on MEPs was aimed at disclosing the cortical somatotopy of oro-facial and hand-arm representations in M1 and vPM. However, to be sure that the MEPs evoked in oro-facial muscles by stimulation of vPM was not die to spread of current to M1, in turn eliciting the response, the MEPs latency was calculated. Anatomical studies in the non-human primate (Dum and Stick, 1991, 2005) have shown indeed that M1 and the pre-motor areas, including vPM, contribute to corticobulbar and corticospinal tract. However, the vPM projections, in comparison with those of M1, show significant differences in terms of numbers (inferior), size and spinal targets (Shimazu et al., 2004; Borra et al., 2010; Firmin et al., 2014). For example, the electrical stimulation of M1 evokes different descending volleys recordable in the pyramidal tract: an early D-wave, which reflects the direct electrical activation of corticospinal axons (Patton and Amassian, 1954), and subsequent indirect waves, with an approximate inter-wave delay of 1.5 ms, considered to reflect trans-synaptic activation, via cortical interneurons, of corticospinal cells (Amassian et al., 1987). The first descending volleys consistent with D-waves, leads to monosynaptic excitation of spinal motoneurons (Porter and Lemon, 1993; Maier et al., 2002; Shimazu et al., 2004). Conversely, there is no evidence that stimulating vPM gives rise to either a D-wave in the corticospinal tract or early motor responses (Shimazu et al., 2004; Maier et al., 2013), suggesting that the connections of this area with the spinal cord must be indirect and involve, for example, specific cortico-cortical connections with M1, which in turn sends numerous direct projections to spinal motoneurons of muscles (Morecraft et al., 2013). A powerful facilitation exerted by vPM on M1 motor output of hand muscles was clearly demonstrated in the macaque (Cerri et al., 2003; Shimazu et al., 2004), suggesting that the M1 may be a critical hub in mediating vPM motor-related activation of spinal motoneurons (Schmidlin et al., 2008). These studies suggest that if vPM has not direct connections with motoneurons, but there is almost another synapse to activate it, the latency of MEPs evoked by stimulating vPM is longer in relation to that of MEPs evoked by stimulating the M1 region composed by cortico-motoneuronal cells. These latter cells have direct connections with motoneurons. The indirect connection between vPM and motoneurons may well explain, also, the higher intensities needed to elicit a motor output from the vPM compared with M1 (Cerri et al., 2003).

When comparing data recorded in non-human primates with the data obtained in our study, the constraints of the human intraoperative method must be discussed. To begin with, HF-DES was delivered to the cortical surface while in the monkey it is normally delivered intra-cortically. Moreover, while the clinical procedure allowed recording the EMG activity from several oro-facial and hand-arm muscles (both ipsi- and contralateral), it was not possible to monitor descending spinal volleys or directly investigate motoneuronal responses. We compared the latency of MEPs elicited by HF-DES on M1 and vPM in the same set of muscles, both at single subject and at population levels. The main result, in the great majority of patients tested, was that MEPs elicited from vPM had significantly longer latencies than those from M1 (**Fig. 3.5**). This main result was found both for responses obtained with single shocks and with trains of shocks, suggesting that our method of inferring the effective pulse within a train of two to five pulses was reliable. This significant difference in the latency of vPM *vs* M1 MEPs argues against the responses evoked from vPM being due to current spread from M1, but rather suggests that these MEPs originated from vPM itself. When observing the distribution of response latencies of MEPs evoked from M1 and vPM, two different distributions emerged, although with a partial overlap. Among the two areas, small differences in latency (<2 ms) as well as considerably larger differences (up to 5-7 ms) were observed, possibly underlying different pathways mediating responses evoked from the two areas. By considering only the average mean values these nuances may be lost; in addition, the method used to estimate the effective pulse in a train may actually underestimate latency differences. The responses from vPM which had latencies only slightly shorter than those from M1 might be conducted by corticospinal fibres, which are probably more slowly conducting than the fastest M1 fibres (Vigneswaran et al., 2011; Firmin et al., 2014), whereas those with greater latency differences (2.5-4.0 ms) might be mediated by a cortico-cortical interaction with M1, as in the macaque (Shimazu et al., 2004). In any event, as in the macaque monkey, our results speak against a fast, direct excitation exerted by vPM on motoneurons comparable with the output from M1. Given the strong, bilateral and reciprocal connections between vPM and M1, it is essential to investigate the functional properties of the vPM regions that make cortico-cortical connections with M1. MEPs evoked in oro-facial muscles by vPM stimulation showed a significantly longer latency than those elicited from M1. Despite the evidence, in macaques, for direct projections from vPM to the trigeminal and facial motor nuclei innervating the OO and MYLO muscles (Simonyan and Jurgens, 2003), the significantly longer latency observed for MEPs from vPM *vs* M1 suggest that, in humans, direct projections are unlikely to be engaged in the direct control of brainstem nuclei. Recently DTI has been utilized to trace pre-motor descending fibres (Verstynen et al., 2011). Interestingly, the majority of descending fibres originating in vPM, are shown to be located between BA44 (posterior part of Broca's area) and BA3 of primary sensory

cortex, just above the lateral sulcus in a relatively concentrated area. Surprisingly, the cortical region reported by Verstynen and colleagues (2011), as the main pre-motor source of descending fibres, did not coincide with our reconstruction maps plotting the pre-motor sites eliciting a clear motor output The discrepancy between our data and the neuroimaging data challenges the complete reliability of the DTI data and highlights the need to confirm the neuroimaging data with electrophysiological recording, in order to create a reliable human model. It should be pointed out that DTI shows the totality of the corticofugal fibres descending to the level of the cerebral peduncle, without identifying specifically corticobulbar or corticospinal fibres, which actually probably make up just a small percentage of the total corticofugal output (Tomasch, 1969). The constraints of this technique do not yet allow the precise identification of how corticofugal components, originating from the different cortical areas, terminate at brainstem and spinal levels.

Conclusively, the analysis of MEPs latency by distinguishing the oro-facial representation in M1 from that in vPM, allowed to localize in these two cortical regions the starting site to be stimulated during speech tasks.

4.2 COMPARISON OF THE DES-INDUCED INTERFERENCE EFFECTS IN M1, vPM AND BROCA'S AREA

Based on the results, the somatotopic organization of the motor responses can be disclosed only in M1 and vPM, excluding Broca's area, since that it has not motor output. It appears that only M1 and vPM are composed by neurons controlling oro-facial muscles. However, for many years, Broca's area has been considered a cortical area involved in motor control of speech, a role recently significantly challenged by neurophysiological studies (Cerri et al 2015; Flinker et al., 2015) and rather suggested to be exerted in neural control of superior language functions, as the semantic, phonological and syntactic processing (Price 2010; Friederici 2011; Katzev et al., 2013; Bourguignon 2014). However, at present, the exact nature of the role of Broca's area in the complex neural network underlying language remains obscure, particularly in motor control of speech, since no study has yet directly measured the activity of phono-articulatory muscles during a speech task, while, for example, the activity of Broca's area is transiently impaired by electrical stimulation. The transient inactivation of a cortical area/pathway involved in motor control of speech, would induce a significant alteration of the phono-articulatory activity when compared with the natural speech performance. Inside of the project, this approach has been applied on Broca's area and also on vPM and on M1 to investigate "how" these areas control the motor programming of speech.

Despite the absence of a proper motor output, the neurosurgical literature reports a clear effect of DES delivered on Broca's area on speech monitored with clinical inspection, therefore it was mandatory to investigate whether the effect on the phono-articulatory muscles, not emerging as a motor output, could be detected in a more ecological condition, i.e. during speech task performance. Thus, the quantitative analyses of EMG activation in phono-articulatory muscles during speech task performance impaired by intraoperative stimulation of Broca's area, M1 and vPM was performed. Concerning M1 and vPM stimulation, the starting site was the oro-facial representations previously identified (**Fig. 3.4**), then extended beyond this area for clinical needs, while the stimulation of Broca's area, in absence of an oro-facial muscle representation, was applied on the whole area.

Broca's area

For more than 100 years, the motor control of language production has been dogmatically attributed to Broca's area (BA44-45) (Berker et al., 1986), despite no conclusive evidence on its motor properties has ever been provided. The putative direct role of Broca's area in motor control of speech must indeed be shown to result in either the shaping of the activity of M1 or in the independent control

of bulbar motoneurons. In both cases, Broca's area is expected to significantly affect, either direct or indirect via M1, the motoneuronal excitability and, in turn, the activity of phono-articulatory muscles. However, the observation that injuries of sole Broca's area do not result in a motor dysfunction in speech production, but rather in improving mutism (Mohr et al., 1978), raised doubts on its significant role in control of speech motor output and as a consequence on "motor theory of speech perception". Interestingly, the "apraxia of speech", a clear phono-articulatory dysfunction, follows to lesions of vPM, rather than BA44-45, suggesting that the motor role so far attributed to Broca's area is actually accomplished by vPM (New et al., 2015). In the most modern model of speech production (Hickok and Poeppel, 2004, 2007; Papoutsi et al., 2009; Long et al., 2016), the ventral part of BA44 is supposed to compute the phonetic encoding translating the syllable to articulatory gestures (Papoutsi et al., 2009; Long et al., 2016) to be executed by the vPM-M1 output control of phono-articulatory muscles (Hickok, 2012). According to this model, a pure lesion to ventral BA44 would block the speech production by preventing the Broca's area to vPM-M1 phonetic translation. The intraoperative *brain mapping* technique actually allows the opportunity to induce, by delivering direct electrical stimulation, LF-DES, a pure "transient lesion" of BA44 in awake patients performing object picture naming and/or counting tasks. The observed "speech arrest" reported by neurosurgeons during LF-DES stimulation onto Broca's area (Luders et al., 1987; Axelson et al., 2009; Chang et al., 2011, Tate et al., 2014), defined as a "complete interruption of ongoing speech in absence of mouth movements or vocal output of patients", seems strongly coherent with modern linguistic model (Hickok and Poeppel, 2004, 2007; Papoutsi et al., 2009; Long et al., 2016). However, some methodological issues must be discussed. First of all the neurosurgical definition of "speech arrest", referring to the DES-induced arrest of ongoing speech, is rather inaccurate, given that it is never reported whether DES is delivered *during* phono-articulation or *just before* the expected onset of the word pronunciation. In the first condition, DES may interrupt the motor program during its execution (an actual arrest of ongoing speech), while in the second DES might prevent or abort the onset of the motor output. The difference in the two conditions is critical, in that the former would support the involvement of Broca's area in the motor control of speech-gestures, while the latter would exclude it. The intraoperative reports never resolve this issue, which however must be clearly settled with a temporally accurate analysis. Here we investigated, with a quantitative approach in the intraoperative setting, the effect of the LF-DES-induced transient inactivation of Broca's area on the EMG activity of phono-articulatory muscles recorded during object picture naming and/or counting tasks. Results clearly demonstrated that the "speech arrest" cannot be attributed to a direct suppression of the activity of phono-articulatory muscles, as expected when inactivating an area controlling the ongoing motor program. During the stimulation, occurring when the word is expected to be pronounced, all

muscles remain relaxed in resting background condition and the speech never starts (**Fig. 3.8**), while LF-DES delivered during ongoing phono-articulation was totally ineffective. Strongly supported by evidence of the absence of a proper motor output from Broca's area in resting state condition, excluding its inclusion within the properly defined motor areas (see above section "Comparison of the DES-induced interference effects in M1, vPM and Broca's area"), this novel approach allows to exclude a proper involvement of Broca's area in motor control of language. Data here reported can be interpreted in light of the most accredited current linguistic model of speech production (Hickok and Poeppel, 2004, 2007; Papoutsi et al., 2009; Long et al., 2016), suggesting that LF-DES is halting the naming process before the execution of the motor program, preventing the onset of speech, possibly during phonetic translation, rather than arresting it. Despite the recent neurosurgical literature reporting the entire BA44-45 responsive to LF-DES with "speech arrest" (Havas et al., 2015; Chang et al., 2016), in our study the positive sites cluster only in the most ventral-posterior sector, vBA44 (**Fig. 3.23**), suggested as candidate for the phonologic encoding of words (Hickok and Poeppel 2004; 2007; Papoutsi et al., 2010).

Based on this analysis, the term "speech arrest" should be reviewed as "speech prevention", given that LF-DES never blocks the actual ongoing speech.

Despite very fascinating, the linguistic model of speech production (Hickok and Poeppel, 2004, 2007; Papoutsi et al., 2009; Long et al., 2016) pointing to Broca's area role in phonetic translation (Papoutsi et al., 2009; Long et al., 2016) seems to be partially undermined by a careful analysis of the intensity needed to elicit the "speech prevention". Results of the quantitative analysis show that "speech prevention" is an almost *all-or-none* intensity-dependent effect. The intensity of LF-DES (*ThreshI*-LF-DES), inducing a speech disruption when applied on M1 and vPM (see below), was ineffective when applied on vBA44 where higher intensity (*SupraThreshI*-LF-DES) was required to induce the "speech prevention". The hypothesis that vBA44 is similar in function to the other motor cortices but just less excitable, seems simplistic, too "*ad hoc*", and unjustified given the structural cyto-architectonic similarities between BA44 and vPM (Amunts et al., 1999, 2010, 2012, 2013). Our interpretation of this result is instead that "speech prevention" might actually result from inactivation of subcortical fibers running below the cortical site of stimulation, explaining why the effect is not elicited with *ThreshI*-DES acting on the cortex but not strong enough to reach the subcortical fibers. Consistently, we report that DES delivered subcortically to vBA44 indeed induces the same effect as *SupraThreshI*-DES delivered cortically. The conceptual consequences of this hypothesis depend on the nature of the axons running subcortical to vBA44. If these fibers are in direct connection with Broca's area, afferents or efferents, then "speech prevention" can still be attributed, although indirectly, to Broca's area, as historically reported, though with the important new evidence that

prevention does not derive from a motor impairment. Conversely, if the stimulated axons are running on their way to other targets bypassing vBA44, the attribution of the "speech prevention" to Broca's cortex, and consequently and more importantly, its role in phonetic translation, must be significantly reconsidered. The system of fibers belonging to the language network and reaching the frontal lobe have been described with the most advanced neuroimaging techniques (Catani et al., 2013). The most relevant tracts are the arcuate *fasciculus* (AF), the superior longitudinal *fasciculus* component II and III (SLF II-III) and the frontal aslant tract (FAT), reported to be involved in different functions in language network, from phonological (Dick and Tremblay, 2012; Chang et al., 2015; Fujii et al., 2015, 2016), syntactic (Chang et al., 2015; Skeide et al., 2016), reading (Gullick et al., 2015), repetition (Chang et al., 2015) processes, starting mechanisms of speech (Botez and Barbeau, 1971; Kinoshita et al., 2015), to phono-articulation (Chang et al., 2015). Building on this well-established body of knowledge, we utilized HARDI tractography to reconstruct the terminations of the AF, SLF II-III and FAT tracts (Fig. 3.25) and disclose whether these subcortical fibers that we think were stimulated by DES, are part of Broca's connectivity (we used the tractography technique to analyse what fibers ran below Broca's area, since it represents the unique instrument that we could used in this experimental setting). Our analysis, consistent with the most recent post-mortem microdissection studies (Lemaire et al., 2013; Sarubbo et al., 2016) shows parallel branches of AF, SLF II-III and FAT terminating in both BA44 and in the neighboring vPM, and running below the sites of stimulation in BA44. All these subcortical systems could be potentially responsible for the "speech prevention" effect, although current spread might not reach the FAT tract as easily, due to its medial and deep course (Sarubbo et al., 2016). Moreover, the observation that DES on vPM induces speech motor disruption (Cerri et al., 2013), and not "speech prevention", suggests that the vBA44 branches, rather than the vPM branches, are likely to be involved. DES of both the AF and SLF vBA44 branches could well interrupt the input feeding Broca's area by interrupting transmission through their axons on their way to Broca's and/or by interrupting elaboration within Broca's area itself. Should this be the case however, the *ThreshI*-DES stimulation of Broca's cortex, i.e. of the final target of the vBA44 branches, should also have caused speech prevention; instead, in all our tests Broca's area was never affected by *ThreshI*-DES. The same argumentation applies to a parallel alternative hypothesis, namely the involvement of U-fibers (Lemaire et al., 2013), also considered possible candidates for phonetic translation in current linguistic theory. U-fibers, not reconstructed here by tractography, are known to originate in BA44 and run superficially to vPM and therefore could be reached by DES. It is evident from this discussion that at the present time the complexity of the language network does not allow an easy allocation of function to all the different structures involved, but it is also evident that the univocal attribution of phonetic translation to Broca's area alone must be revisited.

Conclusively, this approach applied to Broca's area is the first to provide a quantitative analysis of muscular activation before and during ongoing speech and DES of Broca's area. Results exclude a direct role of Broca's area in motor control of speech and shed light on the confounding neurosurgical literature discussing the role of BA44-vPM-M1 circuit in language, previously based exclusively on intraoperative neuropsychological reports. Rather than inducing the interruption of an ongoing speech, i.e. "speech arrest", the intraoperative stimulation of the ventral BA44 results in a complete lack of muscle activation, better defined as "speech prevention". The high DES intensity needed to obtain such "speech prevention" challenges the involvement of Broca's cortex alone in the genesis of the effect, and rather suggests that subcortical fibers, probably the SLF II-III and/or the AF running below vBA44 and reaching both vBA44 and vPM, might also be stimulated by current spread. Our hypothesis therefore considers "speech prevention" as the result of a massive shut down of phonetic encoding, affecting both object picture naming and counting tasks. The possibility that more than one tract running below vBA44 contributes to the effect cannot be ruled out by using only the tractography, neither combining it with electrophysiological reported-data, since that the tractographic technique is only a probabilistic method to estimate what fibers are part of Broca's connectivity. However, it is not possible to perform brain microdissections. Moreover, if more than one tract running below vBA44 contributed to speech prevention effect, also the univocal attribution of phonetic translation to Broca's area should be reconsidered.

vPM

The involvement of vPM in motor control of speech has been demonstrated by several studies (Indefrey and Levelt, 2004; Brown et al., 2008; Bouchard et al., 2013; Tate et al., 2014; Tomasino et al., 2014; Chang et al., 2015; New et al., 2015). However, in order to investigate the distinguishing role of vPM in speech, it is firstly mandatory to precisely localize vPM in human cortex (very clear in non-human primates (Dum and Strick, 2005; Boudrias et al., 2010a,b)). The absence of a clear anatomical border separating the two areas in human brain leads in a difficult interpretation of neuroradiological data showing the involvement of the pre-central sulcus to be ascribed M1 and/or to vPM. Data collected in the first part of the project allowed to disclose the functional distinguishing properties of vPM oro-facial and hand-arm representation (**Fig. 3.4**) and to distinguish vPM motor responses to M1 motor responses. These data were needed to be rigorous in investigating the effect of vPM and M1 stimulation in motor control of speech production.

In 45 patients, vPM was exposed and LF-DES was applied on vPM oro-facial representation and, due to clinical needs, extended to the more ventral portion of vPM, where no motor responses could be evoked (**Fig. 3.4**). LF-DES induced two different speech perturbations: vocalization-like and dys-phono-articulation effects, both suggesting an impairment of the motor program driving the phono-articulatory muscles during both speech tasks. During stimulation, the vocal output of speech was never abolished while the patients kept on trying to articulate the words. These effects are extremely different from the "speech prevention" following Broca's area stimulation and from the "speech arrest" following M1 stimulation.

The vocalization-like effect, similar to those reported by Penfield and Boldrey (1937), occurs simultaneously with a motor disruption in recorded-muscles. A careful scrutiny of the literature suggests a possible explanation of the vocalization-like interferences. In a lesion study, Ziegler and Aichert (2015) published a probabilistic model of speech errors in patients affected by apraxia of speech (AoS), a condition strongly associated with lesions of vPM (see Introduction). They found, among possible speech errors, that consonants are more likely to be compromised than vowel and that the beginning of words is intrinsically likely to be compromised than the other portions of the same words. These data led us to speculate that vocalizations-like effects we observed could be similar to errors in some patients affected by AoS and the result of the patient's inability to correctly load an articulatory program for a consonant at the beginning of a word and to remain 'stuck' on the first vowel. Despite very fascinating this hypothesis need to be demonstrated with a more rigorous analysis based on purposefully designed tests (i.e. planned sequences of words) to be used during intraoperative mapping of vPM (Ziegler and Aichert, 2015). Moreover, we can speculate that this vocalization-like effect may be induced by a transient inactivation of an area involved in speech production rather than interfering with an area involved in vocalization. The human vocalization is indeed generated by the emotional motor system, in which the mesencephalic periaqueductal grey (PAG) plays a central role, as demonstrated by the fact that lesions in the PAG lead to complete mutism in cats, monkeys, as well as in humans. The PAG receives strong projections from higher limbic regions and from the anterior cingulate, insula, and orbitofrontal cortical areas (Schulz et al., 2005) and in turn, projects to the caudal medullary retroambiguus nucleus driving the motoneurons involved in vocalization, i.e., the motoneuronal cell groups innervating soft palate, pharynx, and larynx as well as diaphragm, intercostal, abdominal, and pelvic floor muscles. Together they determine the intrabdominal, intrathoracic, and subglottic pressure needed to generate sounds. Following the production of laryngeal sounds, these are articulated into words. Phono-articulation is controlled by the motor cortex, which, via corticobulbar fibers, has direct access to the motoneurons innervating the muscles of face, mouth, tongue, larynx, and pharynx. In light of this, humans

generates speech by activating two motor systems: the vocalization, by activating the prefrontal-PAG-nucleusretroambiguus-motoneuronal pathway, and, at the same time, the phono-articulation modulating the laryngeal sounds into words and sentences by activating the corticobulbar fibers oro-facial phono-articulatory muscles (Holstege and Subramanian, 2016). Our result, showing a persistent vocalization, suggests that vPM plays a role in motor programming of speech and not in vocalization.
The second recorded-effect was the dys-phono-articulation. The effect can be described as the patients' attempts to articulate a word and the failure to properly do so. This effect was always characterized by a non-clear vocal output and an inadequate motor articulation of speech, called disarticulation of phono-articulatory muscles (dys-phono-articulation). This effect is *similar* to the dysarthria observed in many neurosurgical studies (Petrovich et al., 2005; Tate et al., 2014), but not identical (see below).
Conclusively, we can suggest that both interference-effects are coherent with the functional role attributed to vPM in modern model of speech production (Hickok and Poeppel 2004, 2007; Hickok, 2012), suggesting it to be associated with motor programming of speech and not in generation of the vocalization. The distinguishing features of the effect for vPM and Broca's stimulation during tasks, allows to recognize, in intraoperative setting, the vPM from the anterior neighbouring Broca's area.

Interesting is the observation that the two effects appreciated by clinical inspection -dys-phono-articulation and vocalization-like effects- actually match with three muscular interference-patterns (**Fig. 3.9, 3.10, 3.11**) and none of them have a univocal correlation with the two vocal effects. Accordingly, in neurosurgical literature, it can be reasonably suggested that the sole vocal outcome is again not a precise tool to explore the effect of LF-DES over speech production, while the EMG analysis is a more sensitive monitoring tool.
The three EMG interference-patterns observed (*Clonic-like* pattern, *Pure Inhibitory* and *Inhibitory/ Recruiting* patterns) deserve a proper discussion. All patterns can be defined as disruptions of the proper spatial and temporal coordination of the phono-articulatory muscles, required to perform the fine and highly complex activity of the speech articulation to correctly produce the words clearly supporting the involvement of vPM in motor programming of speech. From a first observational analysis of the EMG recordings, it might be suggested that the vPM neurons coordinate *the phono-articulatory activity* by programming *gestures* organized upon decreasing and increasing the recruitment of different muscles, rather than controlling single muscles in assort of "vocabulary of phono-articulatory actions (or gestures)" similar to the vocabulary of actions suggested for the hand district. The *Pure Inhibitory* pattern clustering in a specific vPM region, the one negative for motor responses (MEPs), suggests the presence of a cluster of inhibitory neurons controlling the phono-

articulatory muscles during *natural* speech *performance*, and therefore not generating a motor output; otherwise, the interference-effect might be due to perturbation of non-inhibitory neurons, such that the inhibitory action on phono-articulatory muscles is exerted from others neurons. However, the first hypothesis is more accredited since, on this cortical portion of vPM, MEPs are not evoked. Notably the *Clonic-like* pattern, clearly excitatory, seems associated to the action of excitatory neurons. However, responding to low frequency stimulation, the neurons recruit the phono-articulatory muscles in clonic fashion. At present, it is difficutl to speculate on this pattern of recruitment (or dys-recruitment), although we can reasonably exclude this behaviour to be related to epileptic activity, since ECoG signal does not report ictal signals. The *Inhibitory/Recruiting* pattern is, instead, characterized by different effects of excitation and inhibition (*vs* to natural performance) on different muscles. This pattern is, at the difference of the others, strictly in analogy with reports of neurons responding selectively to complex movements and goal-directed grasps of the mouth in the rostral portion of the monkey analogous of human vPM (F5) (Maranesi et al., 2012).

With the quantitative analysis, the motor unit recruitment was characterized during the *natural performance* and during *LF-DES interference* (three EMG recognized-patterns). The variations in motor units recruitment were evaluated in the time domain by means of root mean square (RMS, specifically, calculating mean and peak RMS) and in the frequency domain, by means of power spectrum (PS, specifically, by calculating mean and median frequency and area of the power spectrum), which were analysed in each EMG recording for each muscle. The RMS parameters indirectly reflect the motor units recruitment, which can be increased by increasing the frequency of discharge of an already active unit or by recruitment of larger units, in any case indicating an increase or a decrease of force recruited by the analysed muscle; PS parameters reflect the frequency of discharge of the motoneurons innervating the muscles, which can indicate which population of motor units might be more involved in the activation. The patterns of recruitment of motor units in *LF-DES interferences* were significantly different from those observed in *natural performance* and specific to the qualitatively observed EMG patterns. The *Clonic-like* pattern, characterized by an increase in frequencies and amplitude (*vs* to *natural performance*), suggested the occurrence, during stimulation, of an increase in the number of motor recruited-units and/or an increase in discharge frequency used by motoneurons to activate the phono-articulatory muscles. The *Pure Inhibitory* pattern, characterized by a decrease in frequency and amplitude (*vs* to *natural performance*), suggested an inhibitory action on active motoneurons. The *Inhibitory/Recruiting* pattern, characterized by different effects (excitatory *vs* inhibitory) in different muscles, was possibly in analogy with the complex actions represented in the monkey analogue of vPM, F5.

Very interesting is the localization, within vPM, of the three EMG interference-patterns, clustering in different sector of vPM on the 3D reconstruction of stimulation sites of 25 patients in MNI space (**Fig. 3.23**). Among the three patterns, the one exhibiting the most distinguishing features is clearly the *Clonic-like* pattern, clustering in the most dorsal portion of vPM, right at the anterior border with M1. The quantitative analysis of the *Clonic-like* pattern shows that it is basically a recruitment pattern, never (or almost never) showing inhibitory features. This may lead to the hypothesis that the *Clonic-like* pattern could result from the stimulation of an M1-vPM transition zone and the current spread might actually stimulate the M1 in addition to the vPM, supported by the observation that LF-DES applied on M1 induces a strong increase of muscular activity (**Fig. 3.17**). The other two patterns cluster in different positions within the most ventral portion of vPM. The *Pure Inhibitory* pattern cluster in most ventral portion of vPM, while the *Inhibiting/Recruiting* pattern is located in between the other two. Notably, the first pattern is located where HF-DES did not evoked motor responses, while the second pattern is located where HF-DES induced oro-facial responses. Although statistically different, these three EMG interference-patterns show some similarities and may reflect a functional somatotopy within of vPM, characterized by the anatomic/functional clustering of neurons based on gestures rather than simply on the effectors. This hypothesis is also suggested from interference-action induced by LF-DES on all recorded-muscles, independently by EMG pattern. This result is partially supported by a recent study (Bouchard et al., 2013) conducted in epileptic patients, in which Bouchard and colleagues, using an high-resolution, multi-electrode cortical recording during the production of consonant-vowel syllables, described the dynamic organization of sensory-motor cortex (including not only the pre-central *gyrus*, but also the post-central *gyrus*). According their results, the single recording-electrodes had functional representations of multiple phono-articulators, even if they described a clear preference for individual articulators at single electrodes. The multiple representation of phono-articulators correlates with our results, since that when LF-DES was applied on vPM during single-speech tasks, the stimulation interfered with the activity of more phono-articulatory muscles and suggests that probably vPM, as M1 (Book: "Principles of Neural Science", Kandel, Schwartz, Jessell, Siegelbaum, Hudspeth, the 5th edition, 2014), is characterized by a columnar neuronal organization. In fact, the interference-effect induced by DES with the activity of a muscle was induced by stimulating separated cortical sites, not contiguous, suggesting that the cortex hosts multiple sites of neurons controlling a specific muscle and that these regions are mixed with sites controlling other muscles. However, Bouchard and colleagues, as Simonyan and Brown (2008), revealed a dorsal-ventral arrangement of phono-articulatory muscles (larynx, lips, jaw, tongue and larynx), that instead our results do not support. These different results could be due to a different spatial resolution of the eletrodes: the stimulation

electrodes have a major diameter in relation to those of recording, so the first act on a major number of neuronal columnars with different innervation targets, while the latter record a less number of columnars. However, we record the EMG-interferences related to a specific muscle for all the length of vPM, without statistical prevalence, and moreover the different EMG interference-patterns suggest that probably the neurons controlling a specific muscle are functionally organized along all vPM, so we can exclude that a clear muscular somatotopy can be on vPM.

The localization of the differnt interference-effects on the ongoing EMG activity deserves a discussion in light of the neurosurgical literature, taking into account that in almost all the others neururgical setting the EMG activity is not recorded. Thus the comparison among interference-effects and stimulation sites is not possible. The dysarthric effect (see Introduction) was located by Tate and colleagues (2014, **Fig. 1.27**) on more dorsal portion of vPM, where we localize the *Clonic-like* pattern and the more dorsal part of *Inhibiotry/Recruiting* pattern. Moreover, the same studiest (and Sanai et al., 2008) found on the same region, and also on all more ventral portion of vPM, the speech arrest/anarthira phenomenon, which we never record on vPM. Instead, the *Pure Inhibitory* pattern is located on more ventral portion of vPM. In addition to recent neurosurgical literature, we report an interference-effect described, similarly, in 1937 by Penfield and Boldrey: the vocalization-like effect. Although we cannot associate this vocal outcome with a specific EMG interference-pattern, we can compare Penfield and Boldrey (**Fig. 4.1**) stimulation sites with ours. In the figure, the vocalization effects was located on dorsal portion of vPM and on ventral portion of M1. However, we find the

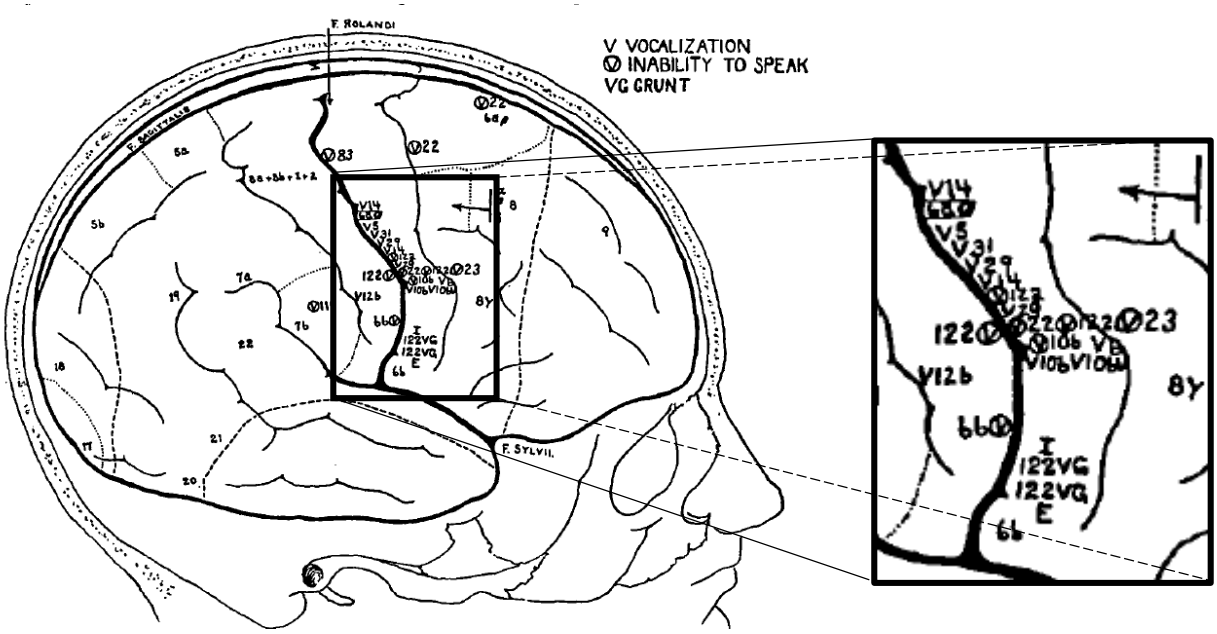

Fig. 4.1 Vocalization map.

From *Penfield and Boldrey, 1937*

analogous vocal effect (called vocalization-like) on all vPM and not on M1. Moreover, Penfield and Boldrey recognized sites where the stimulus induced an inability to speak, or else patients hesitated to denominate a word, but without stop it completely. This effect could be associated to our dys-phono-articulatory interferences, which we find on whole length of vPM, and not only on dorsal portion of vPM (see **Fig. 4.1**). By observing their map, we can highlight also as their vocal outcomes are mixed (vocalization and inability to speak) on cortical surface, analogously to ours (vocalization-like and dys-phono-articulation), even if it is not clear where is the anatomo-functional border between M1 and vPM.

Another issue to be discussed is the effect on two different tasks: object picture naming and counting. As main result, the LF-DES induced the same EMG interference-patterns independently from task. In fact, the tasks are very different, because in the case of object picture naming task, when the image is represented on pc screen, the subject recognizes the depicted object and selects an appropriate concept, or "lemma". After, the range of operations involved in form encoding considers the access to the word's phonological code and ends while the word is being articulated (Indefrey and Levelt 2004). Instead, the counting task is a learned-sequence. However, when the stimulus was applied on the same site during both tasks, the stimulus interfered analogously with the phono-articulatory activity of each muscle (contra- and ipsilateral). This result suggests that vPM may be considered as a common hub activated to coordinate the phono-articulatory activity, possibly via M1, independently from requested tasks. This hub coordinates the contra- and ipsilateral muscles to stimulated hemisphere. In fact, in all our cases, when the LF-DES was applied along vPM interfered with phono-articulatory activity of contra- and ipsilateral recorded-muscles. This data suggests that vPM, of the dominant hemisphere for the language (100% of our instances), programs the activity of contra- and ipsilateral muscles to stimulated hemisphere, without a statistical prevalence. It remains obscure whether the vPM in non-dominant hemisphere exerts a bilateral control or whether language dominance has to be referred also to vPM extending the classical view of the language dominance based on the location of Broca's area. Interstingly enough, the sole asymmetry in bilateral control with a clear preference for the contralateral side, was observed in *Clonic-like* pattern, similarly to M1 effect. The symmetric bilateral effect is supported by studies by Morecraft and colleagues (2001, 2004), reporting vPM projects mainly to the lateral sub-nucleus (containing motoneurons to perioral muscles) on both sides, in particular to spinal trigeminal nucleus, the solitary tract nucleus and the facial nucleus. Differently, the prevalent effect on contralateral muscles observed in the *Clonic-like* pattern, might be due to the stimulation of the M1-vPM transition zone. In this area, the current might spread on M1 in addition to the vPM. M1 controls prevalently the contralateral muscles to hemisphere

(Cattaneo and Pavesi, 2014). Jenny and Saper (1987), by employing the horseradish peroxidase in *Macaca*, indicated that most cortico-nuclear projections of M1 were directed to the lateral and intermediate sub-nuclei on both sides with an ipsilateral:contralateral ratio of around 1:3. Some years later, Morecraft and colleagues (2001, 2004) indicated that M1, together to vPM, was the origin of the majority of cortico-facial fibres. M1 was mainly connected to the contralateral lateral sub-nucleus and therefore contained mainly a representation of the contralateral perioral muscles.

A dedicated discussion deserves the apraxia of speech (AoS) syndrome, in all resembling the effect of the LF-DES on vPM. The AoS is a speech disorder associated with inefficiencies in the translation of speech sounds (phonemes) into the kinematic parameters associated with speech production. It is characterized by changes in speech rate (prolongation of speech sounds/segments and between sound or segment gaps), distorted sounds, consistency in error type, and abnormal prosody (de-stressing of typically stressed syllables and sounds) (Dronkers et al., 1996; Wise et al., 1999; Hillis et al., 2004; Maas et al., 2008; Ackermann and Riecker 2010, Hickok, 2014; New et al., 2015; Ziegler and Aichert, 2015). AoS is historically associated with lesions affected the left vPM (Fox et al., 2001; Hillis et al., 2004; Josephs et al., 2006; Graff-Rafford et al., 2014), but recently New and colleagues (2015) suggested that the bilateral pre-motor cortex is critically associated with AoS. Based on our results and on these studies, we can conclude that interference-effects induced by DES, when applied on vPM, suggest that vPM can to be damaged in patients affected by AoS.

M1

The motor control of speech requires the highly skilled voluntary activation of up to 100 phono-articulatory muscles driven by the bulbar motoneurons. These motoneurons are controlled directly and bilaterally by M1 (Ackermann and Riecker, 2010). The portion of M1 controlling the oro-facial muscles (McGuinness et al., 1980; Sessle and Wiesendanger, 1982) is localized ventrally (Kuypers, 1958b; Morecraft et al., 2014) with respect to the hand spot. This region, as well as that of monkey and chimpanzee, contains the pyramidal motoneurons with direct connections with the pre-motor interneurons and with lower motoneurons in the brainstem (in pontine and medullary lateral tegmental field, Kuypers, 1958a; Holstege and Subramanian, 2015), which innervate, in human, the oro-facial muscles, but also the principle phono-articulatory muscles: mouth, lip, larynx, pharynx, and tongue muscles. The oro-facial muscles are represented in M1 in a dorsal-ventral fashion: face and throat, mouth and lip, larynx and pharynx, and tongue muscles (Penfield and Boldrey, 1957; Simonyan et al., 2011). The same bilateral (although with predominance of contralateral representation)

somatotopic organization was confirmed in transcranial magnetic stimulation (TMS, Cattaneo and Pavesi, 2014) studies, also reporting a relative prevalence of perioral muscles and *orbicularis oris* – that belong to phono-articulatory muscles- on other muscles as *orbicularis oculi* and *frontalis* muscles, not involved in phono-articulatory gestures. Direct cortical connections to motoneurons controlling the phono-articulatory muscle activity have been suggested (Liscic et al., 1998), but still debated.

In our patients (N = 10) the stimulation of the M1 oro-facial representation, with the *ThreshI*-LF-DES during speech tasks, induced "speech arrest", a phenomenon characterized by an absence of the vocal output, due to the visible tetanic contraction of several phono-articulatory muscles, making the articulation of words impossible for the whole duration of the stimulation. When the stimulus was removed, the phono-articulatory function was immediately restored and the patients denominated correctly the word. This motor interference was *similar* to effects defined by Tate and colleagues (2014) and by Deletis and colleagues (2014). The latter added that the contraction was "presumably" accompanied with dysphonic/aphonic speech, while the other studies did not describe the vocal output. However, in our cases, the contraction was always associated with the absence of vocal output. This "speech arrest" phenomenon is very different from the speech arrest occurring during Broca's area stimulation (Tate et al., 2014), or now known as "speech prevention", since when M1 is stimulated, the *natural* speech *performance* is actually "arrested" following to a widespread tetanic recruitment of the recorded phono-articulatory muscles. LF-DES induces indeed a tetanic recruitment of antagonist muscles: at physiological level, the *orbicularis oris* and the *mentali*s are responsible of the lips closure and lengthening of vocal tract, while the *mylohyoid* and the *platysma* of the lips opening and shortening of vocal tract. Instead, the stimulation recruits together these muscles.

The **Figure 3.26**, panel A, shows that the oro-facial M1 representation exposed on the convexity of the cortex is a small region. However, the oro-facial M1 representation obtained with HF-DES and the speech M1 representation obtained with LF-DES, at rest and by interfering with speech tasks, respectively, are superimposed, suggesting that here are located neurons controlling all movements/activity requiring the activation of oro-facial muscles, independently from the goals of the actions to be executed. This area appears too small when considering the functional relevance of phono-articulatory gestures. This is possibly explained by postulating that the remaining part of oro-facial M1 is located inside of the bank of the central sulcus (Geyer et al., 1996; Rathelot and Strick, 2009) and therefore neglected by the brain mapping, exploring mainly the cortical convexity.

4.3 NEUROSURGICAL TERMINOLOGY RELATED TO LF-DES INTERFERENCES DURING SPEECH PRODUCTION

An important issue to be discussed is the terminology routinely used in neurosurgical literature, which is, in light of our results, very confusing. Two are the main confounding elements, the first being the lack of an univocal terminology in the different studies, the second being the very confusing cortical attribution of the effects of DES in the different areas.

LF-DES applied on Broca's area with same threshold intensity of vPM and M1 (*ThreshI*-LF-DES) does not induce a speech interference during the linguistic performances. However, by increasing the stimulation intensity (*SupraThreshI*-LF-DES), it halts the naming/counting process before of the execution of the motor program, preventing the onset of speech rather than arresting it. Despite the recent neurosurgical literature reporting the entire Broca's area (BA44-45) responsive to LF-DES with a phenomenon called "speech arrest" (Havas et al., 2015; Chang et al., 2016), in our study the positive sites cluster only in the most ventral-posterior sector, vBA44, suggested as candidate for the phonologic encoding of words (Hickok and Poeppel 2004; 2007; Papoutsi et al., 2010). Based on this analysis, the term "speech arrest" should be reviewed as "speech prevention", given that LF-DES does not block the actual ongoing speech, but rather prevents its onset.

LF-DES applied on vPM induces two different vocal outputs: dys-phono-articulations and vocalizations-like. These two outputs correspond to three EMG interference-patterns, distributed in direction dorsal-ventral: *Clonic-like* pattern, *Inhibitory/Recruiting* pattern and *Pure Inhibitory* pattern. Thus, it can be reasonably suggested that the sole vocal outcome is not a precise tool to explore the effect of LF-DES in speech production, while the EMG analysis is a more sensitive monitoring tool. Given that in clinical practice, the effect of LF-DES on vPM, i.e. the dys-phono-articulations and vocalizations-like are clearly different from the effect of LF-DES on Broca's area inducing "speech prevention" -occurring without muscle contraction- and the M1 "speech arrest" -with tetanic contraction-, the clinical evaluation of the occurrence of oro-facial movements and the vocal output could be sufficient to distinguish vPM from Broca's area and from M1.
This, however, can be reliable only if the neurosurgeons agree on an univocal attribution of LF-DES effect on vPM. The recent neurosurgical literature is indeed very confusing associating, in different studies, two different interference-effects to vPM: the anarthria/speech arrest or the dysarthria. Tate and colleagues (2014) and sometimes Deletis and colleagues (2014) refer to the term *dysarthria* to as dys-phono-articulation (almost always preserved speech output with articulatory impairment) occurring when the stimulation was applied on pre-central *gyrus*, involving both vPM and M1, while

our data related to dys-phono-articulations are only related to entire vPM. Differently Chang and colleagues (2011) reported the anarthria/speech arrest phenomenon when stimulating vPM (Chang et al., 2011; Tate et al., 2014), an effect never observed in our study when LF-DES was applied on vPM.

LF-DES applied on M1 actually induces an arrest of speech caused by the inability to articulate due to a tetanic muscular contraction. This effect is analogous to that described by Tate and colleagues in 2014 (facial muscle contraction) although with the wrong definition of *dysarthria* (Deletis et al., 2014; Tate et al., 2014).

5. *Conclusion*

In summary, we can conclude that three distinguishing effects are elicited by intraoperative stimulation of Broca' (BA44-45), ventral pre-motor (vPM/vBA6) and primary motor (M1/BA4) cortices during two different speech tasks –object picture naming and counting- both requiring the articulation of single words: LF-DES on Broca's area, particularly on the ventral portion of BA44, induces "speech prevention". LF-DES on vPM induces always a disruption of motor programming –characterized by three EMG interference-patterns- and an alteration of vocal output –characterized by dys-phono-articulations and vocalizations-like effects-. Finally, LF-DES on M1 induces the properly defined "speech arrest", by blocking the ongoing speech following to a tetanic muscular contraction, accompanied by an absence of vocal output.

These results allowed to exclude a role of Broca's area in motor control of speech. In fact, LF-DES *prevents* completely the speech output, otherwise it has not effect on language production. Moreover, the high LF-DES intensity needed to obtain the "speech prevention" (*SupraThreshI*-LF-DES) challenges the involvement of Broca's cortex in the genesis of the effect, rather suggesting it to be related to the subcortical fibers, probably the SLF II-III (superior longitudinal *fasciculus*, II and III components) and/or the AF (arcuate *fasciculus*), running below vBA44 and reached by current spread. LF-DES applied onto subcortical fibers induces the same effect of speech prevention. Besides excluding Broca's area in motor control of speech, these data raise doubts on the distinguishing role of Broca's area in phonetic encoding of single words -attributed specifically to vBA44-, which could occur bypassing Broca's area. The significant correlation of the activation of Broca's area with semantic processing of entire sentences, rather points to its involvement in syntactic processing rather than single words coding. Instead, the results obtained when LF-DES is applied on vPM and M1, suggest that these two areas to play a role in motor control of speech. The stimulation on vPM ever produces an improper articulation of speech, confirming that vPM has a role in motor programming and furthermore acting on contra- and ipsilateral muscles in relation to stimulated hemisphere. Moreover, the three EMG interference-patterns have a clear cortical somatotopy, suggesting that vPM is not characterized by muscular somatotopy, although by a behavioural somatotopy. While, the "speech arrest" induced when the LF-DES is applied on M1, confirms that M1 controls directly –by corticobulbar tract- the phono-articulatory muscles.

The interference-effects, which we recognize, are different from that of the recent intraoperative literature, probably because in literature it has not been not clearly reported so far a reliable

methodology to identify the functional border between the M1 and vPM oro-facial representations and because in intraoperative setting the phono-articulatory activity is recorded only in very few teams. In this study, we report how to functionally disclose, by means of DES, the functional border between vPM and M1. Moreover, by recording the activity of some phono-articulatory muscles, we report how to distinguish, based on EMG specific features, among Broca's area, vPM and M1 and to improve the knowledge related to played-role by three areas in speech production.

The data related to anatomical and functional border between Broca's area, vPM and M1 have been published in two scientific papers: Cerri and colleagues (2015) and Fornia and colleagues (2016). The neuro-physiological data obtained by stimulating Broca's area during speech tasks have been submitted to a scientific journal for the publication. While those related to healthy subjects and to stimulation of vPM and M1 during speech tasks are in preparation for the submission to the other journals.

6. *References*

AbdulSabur NY, Xu Y, Liu S, Chow HM, Baxter M, Carson J, Braun AR. Neural correlates and network connectivity underlying narrative production and comprehension: a combined fMRI and PET study. *Cortex*. 2014. 57: 107-127.

Ackermann H, Riecker A. The contribution(s) of the insula to speech production: a review of the clinical and functional imaging literature. *Brain Structure and Function*. 2010. 21: 419-433.

Allison T, McCarthy G, Luby M, Puce A, Spencer DD. Localization of functional regions of human mesial cortex by somatosensory evoked potential recording and by cortical stimulation. *Electroencephalography and Clinical Neurophysiology*. 1996. 100: 126-140.

Amassian VE, Stewart M, Quirk GJ, Rosenthal JL. Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery*. 1987. 20: 74-93.

Amunts K, Schleicher A, Bürgel U, Mohlberg H, Uylings HB, Zilles K. Broca's region revisited: cytoarchitecture and intersubject variability. *Journal of Comparative Neurology*. 1999. 412: 319-341.

Amunts K, Lenzen M, Friederici AD, Schleicher A, Morosan P, Palomero-Gallagher N, Zilles K. Broca's region: novel organizational principles and multiple receptor mapping. *PLoS Biol*ogy. 2010. 8. pii: e1000489. doi: 10.1371/journal.pbio.1000489.

Amunts K, Zilles K. Architecture and organizational principles of Broca's region. *Trends in cognitive sciences*. 2012. 16: 418-426.

Andersen RA. Multimodal integration for the representation of space in the posterior parietal cortex. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 1997. 352: 1421-1428.

Ardila A. A Proposed Neurological Interpretation of Language Evolution. *Behavioural Neurology*. 2015. doi: 10.1155/2015/872487.

Axelson HW, Hesselager G, Flink R. Successful localization of the Broca area with short-train pulses instead of 'Penfield' stimulation. *Seizure*. 2009. 18: 374-375.

Baker E, Blumstein SE, Goodglass H. Interaction between phonological and semantic factors in auditory comprehension. *Neuropsychologia*. 1981. 19: 1-15.

Barinaga M. Remapping the motor cortex. *Science*. 1995. 268: 1696-1698.

Beckman RJ, Nachtsheim CJ, Cook RD. Diagnostics for Mixed-Model Analysis of Variance. *Technometrics*. 1987. 29: 413-426.

Beckers GJL, Bolhuisb JJ, Okanoya K, Berwick RC. Birdsong neurolinguistics: songbird context-free grammar claim is premature. *Neuroethology*. 2012. 23: 139-145.

Bello L, Gallucci M, Fava M, Carrabba G, Giussani C, Acerbi F, Baratta P, Songa V, Conte V, Branca V, Stocchetti N, Papagno C, Gaini SM. Intraoperative subcortical language tract mapping guides surgical removal of gliomas involving speech areas. *Neurosurgery*. 2007. 60: 67-82.

Bello L, Riva M, Fava E, Ferpozzi V, Castellano A, Raneri F, Pessina F, Bizzi A, Falini A, Cerri G. Tailoring neurophysiological strategies with clinical context enhances resection and safety and expands indications in gliomas involving motor pathways. *Neuro Oncology*. 2014. 16: 1110-1128.

Berker EA, Berker AH, Smith A. Translation of Broca's 1865 report localization of speech in the third left frontal convolution. *Archives of neurology*. 1986. 43: 1065-1072.

Berwick RC, Okanoya K, Beckers GJ, Bolhuis JJ. Songs to syntax: the linguistics of birdsong. *Trends in Cognitive Science*. 2011. 15:113-121.

Berwick RC, Friederici AD, Chomsky N, Bolhuis JJ. Evolution, brain, and the nature of language. *Trends in Cognitive Sciences*. 2013. 17:89-98.

Berwick RC, Chomsky N. Why only us. Language and evolution. MIT Press. 2016

Bickerton D. Language evolution: A brief guide for linguists. *Lingua*. 2007. 117: 510-526.

Binkofski F, Buccino G, Posse S, Seitz RJ, Rizzolatti G, Freund H. A fronto-parietal circuit for object manipulation in man: evidence from an fMRI-study. *European Journal of Neuroscience*. 1999. 11: 3276-3286.

Bishop DV, Adams C. A prospective study of the relationship between specific language impairment, phonological disorders and reading retardation. *Journal of Child Psychology and Psychiatry*. 1990. 31: 1027-1050.

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

Bolhuis JJ, Okanoya K, Scharff C. Twitter evolution: converging mechanisms in birdsong and human speech. *Nature Review Neuroscience*. 2010. 11:747-759.

Bonini L, Serventi FU, Simone L, Rozzi S, Ferrari PF, Fogassi L. Grasping neurons of monkey parietal and premotor cortices encode action goals at distinct levels of abstraction during complex action sequences. *The Journal of Neuroscience*. 2011. 31:5876-5887.

Borra E, Belmalih A, Gerbella M, Rozzi S, Luppino G. Projections of the hand field of the macaque ventral premotor area f5 to the brainstem and spinal cord. *The Journal of Comparative Neurology*. 2010. 518: 2570-2591.

Botez MI, Barbeau A. Role of subcortical structures, and particularly of the thalamus, in the mechanisms of speech and language. A review. *International Journal of Neurology Research*. 1971. 8: 300-320.

Bouchard KE, Mesgarani N, Johnson K, Chang EF. Functional organization of human sensorimotor cortex for speech articulation. *Nature*. 2013. 495: 327-332.

Boudrias MH, Lee, Svojanovsky S, Cheney PD. Forelimb muscle representations and output properties of motor areas in the mesial wall of rhesus macaques. *Cerebral Cortex*. 2010a. 20: 704-719.

Boudrias MH, McPherson RL, Frost SB, Cheney PD. Output properties and organization of the forelimb representation of motor areas on the lateral aspect of the hemisphere in rhesus macaques. *Cerebral Cortex*. 2010b. 20: 169-186.

Bourguignon NJ. A rostro-caudal axis for language in the frontal lobe: the role of executive control in speech production. *Neuroscience & Biobehavioral Reviews*. 2014. 47: 431-44.

Brochier T, Spinks RL, Umilta´ MA, Lemon RN. Patterns of muscle activity underlying object-specific grasp by the macaque monkey. *Journal of Neurophysiology*. 2004. 92: 1770-1782.

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

Bucci M, Mandelli ML, Berman JI, Amirbekian B, Nguyen C, Berger MS, Henry RG. Quantifying diffusion MRI tractography of the corticospinal tract in brain tumors with deterministic and probabilistic methods. *Neuroimage: Clinical*. 2013. 3:361-368.

Buccino G, Vogt S, Ritzl A, Fink GR, Zilles K, Rizzolatti G. Neural circuits underlying imitation learning of hand actions: An event-related fMRI study. *Neuron*. 2004. 42: 323–334.

Cabinio M, Blasi V, Borroni P, Montagna M, Iadanza A, Falini A, Cerri G. The shape of motor resonance: right- or left-handed? *Neuroimage*. 2010. 51: 313-323.

Catani M, Mesulam MM, Jakobsen E, Malik F, Martersteck A, Wieneke C, Thompson CK, Thiebaut de Schotten M, Dell'Acqua F, Weintraub S, Rogalski E. A novel frontal pathway underlies verbal fluency in primary progressive aphasia. *Brain*. 2013. 136: 2619-2628.

Cattaneo L, Pavesi G. The facial motor system. *Neuroscience & Behavioural Review*. 2014. 38: 135-159.

Caverzasi E, Papinutto N, Amirbekian B, Berger MS, Henry RG. Q-ball of inferior fronto-occipital fasciculus and beyond. *PLoS One*. 2014. 9: e100274.

Caverzasi E, Hervey-Jumper SL, Jordan KM, Lobach IV, Li J, Panara V, Racine CA, Sankaranarayanan V, Amirbekian B, Papinutto N, Berger MS, Henry RG. Identifying preoperative language tracts and predicting postoperative functional recovery using HARDI q-ball fiber tractography in patients with gliomas. *Journal of Neurosurgery*. 2016. 125: 33-45.

Cerri G, Shimazu H, Maier MA, Lemon RN. Facilitation from ventral premotor cortex of primary motor cortex outputs to macaque hand muscles. *Journal of Neurophyisology*. 2003. 90: 832-842.

Cerri G, Cabinio M, Blasi V, Borroni P, Iadanza A, Fava E, Fornia L, Ferpozzi V, Riva M, Casarotti A, Martinelli Boneschi F, Falini A, Bello L. The mirror neuron system and the strange case of Broca's area. *Human Brain Mapping*. 2015. 36: 1010-1027.

Chainay H, Krainik A, Tanguy ML, Gerardin E, Le Bihan D, Lehéricy S. Foot, face and hand representation in the human supplementary motor area. *Neuroreport*. 2004. 15: 765-769.

Chang EF, Wang DD, Perry DW, Barbaro NM, Berger MS. Homotopic organization of essential language sites in right and bilateral cerebral hemispheric dominance. *Journal of Neurosurgery*. 2011. 114: 893-902.

Chang EF, Raygor KP, Berger MS. Contemporary model of language organization: an overview for neurosurgeons. *Journal of Neurosurgery*. 2015. 122: 250-261.

Chang EF, Breshears JD, Raygor KP, Lau D, Molinaro AM, Berger MS. Stereotactic probability and variability of speech arrest and anomia sites during stimulation mapping of the language dominant hemisphere. *Journal of Neurosurgery*. 2016. 19: 1-8.

Chen SHA, Desmond JE. Cerebrocerebellar networks during articulatory rehearsal and verbal working memory tasks. *Neuroimage.* 2005a. 24: 332-338.

Chen SHA, Desmond JE. Temporal dynamics of cerebro-cerebellar network recruitment during a cognitive task. *Neuropsychologia*. 2005b. 43: 1227-1237.

Costafreda SG, Fu CH, Lee L, Everitt B, Brammer MJ, David AS. A systematic review and quantitative appraisal of fMRI studies of verbal fluency: role of the left inferior frontal gyrus. *Human Brain Mapping*. 2006. 27: 799-810.

Coudè G, Ferrari PF, Rodà F, Maranesi M, Borelli E, Veroni V, Monti F, Rozzi S, Fogassi L. Neurons controlling voluntary vocalization in the macaque ventral premotor cortex. *Plos one*. 2011. 6: e26822.

Crosson B, Sadek JR, Maron L, Gökçay D, Mohr CM, Auerbach EJ, Freeman AJ, Leonard CM, Briggs RW. Relative shift in activity from medial to lateral frontal cortex during internally versus externally guided word generation. *Journal of Cognitive Neuroscience*. 2001. 13: 272-283.

Cruccu G, Inghilleri M, Berardelli A, Romaniello A, Manfredi M. Cortical mechanisms mediating the inhibitory period after magnetic stimulation of the facial motor area. *Muscle Nerve*. 1997. 20: 418-424.

D'Andrea E, Barbaix E. Anatomic research on the perioral muscles, functional matrix of the maxillary and mandibular bones. *Surgical and Radiologic Anatomy*. 2006. 28: 261-266.

De Witt Hamer PC, Moritz-Gasser S, Gatignol P, Duffau H. Is the human left middle longitudinal fascicle essential for language? A brain electrostimulation study. *Human Brain Mapping*. 2011. 32: 962-973.

Deletis V, Rogic M, Ferandez-Conejero I, Gabarrròs A, Jeroncic A. Neurophysiologic markers in laryngeal muscles indicate functional anatomy of laryngeal primary motor cortex and premotor cortex in the caudal opercular part of inferior frontal gyrus. *Clinical Neurophysiology*. 2014. 125: 1912-1922.

Démonet JF, Thierry G, Cardebat D. Renewal of the neurophysiology of language: functional neuroimaging. *Physiological Reviews*. 2005. 85: 49-95.

Dick AS, Bernal B, Tremblay P. The language connectome: new pathways, new concepts. *Neuroscientist*. 2014. 20: 453-467.

di Pellegrino G, Fadiga L, Fogassi L, Gallese V, Rizzolatti G. Understanding motor events: a neurophysiological study. *Experimental Brain Research*. 1992. 91: 176-180.

Diogo R, Wood BA, Aziz MA, Burrows A. On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. *Journal of Anatomy*. 2009. 215: 300-319.

Dobbins EG, Feldman JL. Differential innervation of protruder and retractor muscles of the tongue in rat. *Journal of Comparative Neurology.* 1995. 357: 376-394.

Dronkers NF. A new brain region for coordinating speech articulation. *Nature*. 1996. 384: 159-161.

Dronkers NF. The pursuit of brain-language relationships. *Brain and Language*. 2000. 71:59-61.

Dronkers NF, Wilkins DP, Van Valin RD Jr, Redfern BB, Jaeger JJ. Lesion analysis of the brain areas involved in language comprehension. *Cognition*. 2004. 92: 145-177.

Dronkers NF, Plaisant O, Iba-Zizen MT, Cabanis EA. Paul Broca's historic cases: High resolution MR imaging of the brains of Leborgne and Lelong. *Brain*. 2007. 130: 1432-1441.

Dubach P, Guggisberg AG, Rösler KM, Hess CW, Mathis J. Significance of coil orientation for motor evoked potentials from nasalis muscle elicited by transcranial magnetic stimulation. *Clinical Neurophysiology*. 2004. 115: 862-870.

Duffau H, Capelle L, Sichez N, Denvil D, Lopes M, Sichez JP, Bitar A, Fohanno D. Intraoperative mapping of the subcortical language pathways using direct stimulations. An anatomo-functional study. *Brain*. 2002. 125: 199-214.

Duffau H, Gatignol P, Mandonnet E, Peruzzi P, Tzourio-Mazoyer N, Capelle L. New insights into the anatomo-functional connectivity of the semantic system: a study using cortico-subcortical electrostimulations. *Brain*. 2005. 128: 797-810.

Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. *The Journal of Neuroscience*. 1991. 11: 667-689.

Dum RP, Strick PL. Frontal lobe inputs to the digit representations of the motor areas on the lateral surface of the hemisphere. *Journal of Neuroscience*. 2005. 25: 1375-1386.

Edwards E, Nagarajan SS, Dalal SS, Canolty RT, Kirsch HE, Barbaro NM, Knight RT. Spatiotemporal imaging of cortical activation during verb generation and picture naming. *Neuroimage*. 2010. 50: 291-301.

Edwards LJ, Muller KE, Wolfinger RD, Qaqish BF, Schabenberger O. An R2 statistic for fixed effects in the linear mixed model. *Statistics in Medicine*. 2008. 28: 6137–6157.

Ehrsson HH, Fagergren A, Jonsson T, Westling G, Johansson RS, Forssberg H. Cortical activity in precision- versus power-grip tasks: an fMRI study. *Journal of Neurophysiology*. 2000. 83: 528-536.

Ehrsson HH, Fagergren E, Forssberg H. Differential fronto-parietal activation depending on force used in a precision grip task: an fMRI study. *Journal of Neurophysiology*. 2001. 85: 2613-2623.

Fairbanks G. Systematic research in experimental phonetics. I. A theory of the speech mechanism as a servosystem. *Journal of Speech and Hearing Disorders*. 1954. 19: 133-139.

Fedorenko E, Fillmore P, Smith K, Bonilha L, Fridriksson J. The superior precentral gyrus of the insula does not appear to be functionally specialized for articulation. *Journal of Neurophysiology*. 2015. 113: 2376-2382.

Ferrari PF, Gallese V, Rizzolatti G, Fogassi L. Mirror neurons responding to the observation of ingestive and communicative mouth actions in the monkey ventral premotor cortex. *European Journal of Neuroscience*. 2003. 17: 1703-1714.

Fink BR. Complexity. *Science*. 1986. 231: 319.

Firlund AJ, Cacioppo JT. Guidelines for Human Electromyographic Research. *Psychophysiology*. 1996. 23: 107-127.

Firmin L, Field P, Maier MA, Kraskov A, Kirkwood PA, Nakajima K, Lemon RN, Glickstein M. Axon diameters and conduction velocities in the macaque pyramidal tract. *Journal of Neurophysiology*. 2014. 112: 1229-1240.

Fischer U, Hess CW, Rösler KM. Uncrossed cortico-muscular projections in humans are abundant to facial muscles of the upper and lower face, but may differ between sexes. *Journal of Neurology*. 2005. 252: 21-26.

Flinker A, Korzeniewska A, Shestyuk AY, Franaszczuk PJ, Dronkers NF, Knight RT, Crone NE. Redefining the role of Broca's area in speech. *Proceedings of the national academy of sciences*. 2015. 112: 2871-2875.

Fornia L, Ferpozzi V, Montagna M, Rossi M, Riva M, Pessina F, Martinelli Boneschi F, Borroni P, Lemon RN, Bello L, Cerri G. Functional Characterization of the Left Ventrolateral Premotor Cortex in Humans: A Direct Electrophysiological Approach. *Cerebral Cortex*. 2016. [Epub ahead of print]

Fox RJ, Kasner SE, Chatterjee A, Chalela JA. Aphemia: An isolated disorder of articulation. *Clinical Neurology and Neurosurgery*. 2001. 103: 123-126.

Freilinger G, Happak W, Burggasser G, Gruber H. Histochemical mapping and fiber size analysis of mimic muscles. *Plastic and Reconstructive Surgery*. 1990. 86: 422-428.

Fridlund AJ, Cacioppo JT. Guidelines for human electromyographic research. *Psychophysiology*. 1986. 23: 567-589.

Fridriksson J, Fillmore P, Guo D, Rorden C. Chronic Broca’s Aphasia Is Caused by Damage to Broca’s and Wernicke’s Areas. *Cerebral Cortex*. 2015. 25: 4689-4696.

Fried I, Katz A, McCarthy G, Sass KJ, Williamson P, Spencer SS, Spencer DD. Functional organization of human supplementary motor cortex studied by electrical stimulation. *Journal of Neuroscience*. 1991. 11: 3656-3666.

Friederici, A.D. The brain basis of language processing: from structure to function. *Physiological Reviews*. 2011. 91: 1357-1392.

Fucci D, Crary MA, Warren JA, Bond ZS. Interaction between auditory and oral sensory feedback in speech regulation. *Perceptual and Motor Skills*. 1977. 45: 123-129.

Fujii M, Maesawa S, Motomura K, Futamura M, Hayashi Y, Koba I, Wakabayashi T. Intraoperative subcortical mapping of a language-associated deep frontal tract connecting the superior frontal gyrus to Broca's area in the dominant hemisphere of patients with glioma. *Journal of Neurosurgery*. 2015. 122: 1390-1396.

Fujii, M. Maesawa S, Ishiai S, Iwami K, Futamura M, Saito K. Neural basis of language: an overview of an evolving model. *Neurologia Medico Chirurgica (Tokyo)*. 2016. 122: 379-386.

Gainotti G, Miceli G, Silveri MC, Villa G. Some anatomo-clinical aspects of phonemic and semantic comprehension disorders in aphasia. *Acta Neurologica Scandinavica*. 1982. 66: 652-665.

Gallese V, Fadiga L, Fogassi L, Rizzolatti G. Action recognition in the premotor cortex. *Brain*. 1996. 119: 593-609.

Garrett MC, Pouratian N, Liau LM. Use of language mapping to aid in resection of gliomas in eloquent brain regions. *Neurosurgery Clinics of North America*. 2012. 23: 497-506.

Gentilucci M, Fogassi L, Luppino G, Matelli M, Camarda R, Rizzolatti G. Functional organization of inferior area 6 in the macaque monkey. I. Somatotopy and the control of proximal movements. *Experimental Brain Research*. 1988. 71: 475-490.

Gerardin E, Sirigu A, Lehericy S, Pauline J-B, Gaymard B, Marsault C, Agid Y, Le Bihan D. Partially overlapping neural networks for real and imagined hand movements. *Cerebral Cortex*. 2000. 10: 1093-1104.

Gerlach C, Law I, Paulson OB. When action turns into words. Activation of motor-based knowledge during categorization of manipulable objects. *Journal of Cognitive Neuroscience*. 2002. 14: 1230-1239.

Geyer S, Ledberg A, Schleicher A, Kinomura S, Schormann T, Bürgel U, Klingberg T, Larsson J, Zilles K, Roland PE. Two different areas within the primary motor cortex of man. *Nature*. 1996. 382: 805-807.

Geyer S, Matelli M, Luppino G, Zilles K. Functional neuroanatomy of the primate isocortical motor system. *Anatomy and Embryology (Berl)*. 2000. 202: 443-474.

Godschalk M, Mitz AR, van Duin B, van der Burg H. Somatotopy of monkey premotor cortex examined with microstimulation. *Neuroscience Research*. 1995. 23: 269-279.

Graff-Radford J, Jones DT, Strand EA, Rabinstein AA, Duffy JR, Josephs KA. The neuroanatomy of pure apraxia of speech in stroke. *Brain and Language*. 2014. 129: 43-46.

Grafton ST, Arbib MA, Fadiga L, Rizzolatti G. Localization of grasp representations in humans by positron emission tomography. 2. Observation compared with imagination. *Experimental Brain Research*. 1996. 112: 103-111.

Graziano M. The organization of behavioral repertoire in motor cortex. *Annual Review of Neuroscience*. 2006. 29: 105-134.

Graziano MS, Aflalo TN. Mapping behavioral repertoire onto the cortex. *Neuron*. 2007. 56: 239-251.

Grill-Spector K, Kourtzi Z, Kanwisher N. The lateral occipital complex and its role in object recognition. *Vision Research*. 2001. 41: 1409-1422.

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

Gullick MM, Booth JR. The direct segment of the arcuate fasciculus is predictive of longitudinal reading change. *Developmental Cognitive Neuroscience*. (2015) 13: 68-74.

Hagoort, P. On Broca, brain, and binding: a new framework. *Trends in Cognitive Science*. 2005. 9: 416-423.

Hagoort P, Indefrey P. The neurobiology of language beyond single words. *Annual Review of Neuroscience*. 2014. 37: 347-362. doi: 10.1146/annurev-neuro-071013-013847.

Havas V, Gabarrós A, Juncadella M, Rifa-Ros X, Plans G, Acebes JJ, de Diego Balaguer R, Rodríguez-Fornells A. Electrical stimulation mapping of nouns and verbs in Broca's area. *Brain and Language*. 2015. 145-146: 53-63.

Hendry SH, Jones EG. Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortex. *Journal of Neuroscience*. 1981. 1: 390-408.

Henry JD, Crawford JR. A meta-analytic review of verbal fluency performance in patients with traumatic brain injury. *Neuropsychology*. 2004. 18: 621-628.

Hickok G, Poeppel D. Towards a functional neuroanatomy of speech perception. *Trends in Cognitive Sciences*. 2000. 4: 131-138.

Hickok G, Poeppel D. Dorsal and ventral streams: a framework for understanding aspects of the functional anatomy of language. *Cognition*. 2004. 92: 67-99.

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

Hickok G, Okada K, Barr W, Pa J, Rogalsky C, Donnelly K, Barde L, Grant A. Bilateral capacity for speech sound processing in auditory comprehension: evidence from Wada procedures. *Brain and Language*. 2008. 107: 179-184.

Hickok G. Computational neuroanatomy of speech production. *Nature reviews*. 2012a. 13: 135-145.

Hickok G. The cortical organization of speech processing: Feedback control and predictive coding the context of a dual-stream model. *Journal of Communication Disorders.* 2012b. 45: 393-402.

Hickok G. The Myth of Mirror Neurons: The Real Neuroscience of Communication and Cognition. WW Norton and Company. New York London. 2014.

Hickok G, Rogalsky C, Chen R, Herskovits EH, Townsley S, Hillis AE. Partially overlapping sensorimotor networks underlie speech praxis and verbal short-term memory: evidence from apraxia of speech following acute stroke. *Frontiers in Human Neuroscience.* 2014. 8: 649.

Hillis AE, Work M, Barker PB, Jacobs MA, Breese EL, Maurer K. Re-examining the brain regions crucial for orchestrating speech articulation. *Brain*. 2004. 127: 1479-1487.

Hiroshima S, Anei R, Murakami N, Kamada K. Functional localization of the supplementary motor area. *Neurologia Medico Chirurgica (Tokyo).* 2014. 54: 511-520.

Holstege G, Graveland G, Bijker-Biemond C, Schuddeboom I. Location of motoneurons innervating soft palate, pharynx and upper esophagus. Anatomical evidence for a possible swallowing center in the pontine reticular formation. An HRP and autoradiographical tracing study. *Brain Behavior and Evolution*. 1983. 23: 47-62.

Holstege G, Subramanian HH. Two different motor systems are needed to generate human speech. *Journal of comparative neurology*. 2016. 524: 1558-1577.

Hoshi E, Tanji J. Distinctions between dorsal and ventral premotor areas: anatomical connectivity and functional properties. *Current Opinion in Neurobiology*. 2007. 17: 234-242.

Houde JF, Chang EF. The cortical computations underlying feedback control in vocal production. *Current Opinion in Neurobiology*. 2015. 33: 174-181.

Huang CS, Sirisko MA, Hiraba H, Murray GM, Sessle BJ. Organization of the primate face motor cortex as revealed by intracortical microstimulation and electrophysiological identification of afferent inputs and corticobulbar projections. *Journal of Neurophysiology*. 1988. 59: 796-818.

Hultén A, Vihla M, Laine M, Salmelin R. Accessing newly learned names and meanings in the native language. *Human Brain Mapping*. 2009. 30: 976-989.

Iacoboni M, Woods RP, Brass M, Bekkering H, Mazziotta JC, Rizzolatti G. Cortical mechanisms of human imitation. *Science*. 1999. 286: 2526–2528.

Ikeda A, Lüders HO, Burgess RC, Shibasaki H. Movement-related potentials recorded from supplementary motor area and primary motor area. Role of supplementary motor area in voluntary movements. *Brain*. 1992. 115: 1017-1043.

Indefrey P. The spatial and temporal signatures of word production components: a critical update. *Frontiers in psychology*. 2011. 2: 255.

Indefrey P, Levelt, WJ. The spatial and temporal signatures of word production components. C*ognition*. 2004. 92: 101-144.

Ishai A, Ungerleider LG, Martin A, Schouten JL, Haxby JV. Distributed representation of objects in the human ventral visual pathway. *Proceedings of the national academy of sciences*. 1999. 96: 9379-9384.

Jeannerod M, Arbib MA, Rizzolatti G, Sakata H. Grasping objects: the cortical mechanisms of visuomotor transformation. *Trends of Neuroscience*. 1995. 18: 314-320.

Jenny AB, Saper CB. Organization of the facial nucleus and corticofacial projection in the monkey: a reconsideration of the upper motor neuron facial palsy. *Neurology*. 1987. 37: 930-939.

Jezzini A, Caruana F, Stoianov I, Gallese V, Rizzolatti G. Functional organization of the insula and inner perisylvian regions. *Proceedings of the national academy of sciences*. 2012. 109: 10077-10082.

Johnson PB, Ferraina S, Bianchi L, Caminiti R. Cortical networks for visual reaching: physiological and anatomical organization of frontal and parietal arm regions. *Cerebral Cortex*. 1996. 6: 102-119.

Josephs KA, Duffy JR, Strand EA, Whitwell JL, Layton KF, Parisi JE, Petersen RC. Clinicopathological and imaging correlates of progressive aphasia and apraxia of speech. *Brain*. 2006. 129: 1385-1398.

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

Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ. Principles of Neural Science. 5th edition 2014.

Kantak SS, Stinear JW, Buch ER, Cohen LG. Rewiring the brain: potential role of the premotor cortex in motor control, learning, and recovery of function following brain injury. *Neurorehabilitation and Neural Repair*. 2012. 26: 282-292.

Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. 19th edition 2015.

Katzev M, Tüscher O, Hennig J, Weiller C, Kaller CP. Revisiting the functional specialization of left inferior frontal gyrus in phonological and semantic fluency: the crucial role of task demands and individual ability. *Journal of Neuroscience*. 2013. 33: 7837-7845.

Keller A, Asanuma H. Synaptic relationships involving local axon collaterals of pyramidal neurons in the cat motor cortex. *Journal of Comparative Neurology*. 1993. 336: 229-242.

Kent RD. Research on speech motor control and its disorders: a review and prospective. *Journal of Communication Disorders*. 2000. 33: 391-427.

Kinoshita M, de Champfleur NM, Deverdun J, Moritz-Gasser S, Herbet G, Duffau H. Role of fronto-striatal tract and frontal aslant tract in movement and speech: an axonal mapping study. *Brain structure and function.* 2015. 220: 3399-3412.

Kinoshita M, Miyashita K, Tsutsui T, Furuta T, Nakada M. Critical Neural Networks in Awake Surgery for Gliomas. *Neurologia medico-chirurgica (Tokyo).* 2016. 56: 674-686.

Kirby S. The evolution of language. In Dunbar, R. and Barrett, L., editors, *Oxford Handbook of Evolutionary Psychology*. 2007. Pages 669-681.

Klapp ST. Reaction time analysis of two types of motor preparation for speech articulation: action as a sequence of chunks. *Journal of Motor Behavior*. 2003. 35:135-150.

Kobayashi M, Théoret H, Mottaghy FM, Gangitano M, Pascual-Leone A. Intracortical inhibition and facilitation in human facial motor area: difference between upper and lower facial area. *Clinical Neurophysiology*. 2001. 112: 1604-1611.

Krainik A, Lehéricy S, Duffau H, Vlaicu M, Poupon F, Capelle L, Cornu P, Clemenceau S, Sahel M, Valery CA, Boch AL, Mangin JF, Bihan DL, Marsault C. Role of the supplementary motor area in motor deficit following medial frontal lobe surgery. *Neurology*. 2001. 57: 871-878.

Krainik A, Lehéricy S, Duffau H, Capelle L, Chainay H, Cornu P, Cohen L, Boch AL, Mangin JF, Le Bihan D, Marsault C. Postoperative speech disorder after medial frontal surgery: role of the supplementary motor area. *Neurology*. 2003. 60: 587-594.

Kraskov A, Dancause N, Quallo MM, Shepherd S, Lemon RN. Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? *Neuron*. 2009. 64: 922-930.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD. Corticocortical inhibition in human motor cortex. *Journal of Physiology*. 1993. 471: 501-519.

Kuypers HGJM. Some projections from the peri-central cortex to the pons and lower brain-stem in monkey and chimpanzee. *Journal of comparative neurology*. 1958a. 110: 221-255.

Kuypers HGJM. Corticobulbar connexions to the pons and lower brain-stem in men: an anatomical study. *Brain*. 1958b. 81: 364-388.

Kuypers HGJM. An anatomical analysis of cortico-bulbar connexions to the pons and lower brain stem in the cat. Journal of Anatomy. 1958c. 92: 198-218.

Lemaire JJ, Golby A, Wells WM, Pujol S, Tie Y, Rigolo L, Yarmarkovich A, Pieper S, Westin CF, Jolesz F, Kikinis R. Extended Broca's area in the functional connectome of language in adults: combined cortical and subcortical single-subject analysis using fMRI and DTI tractography. *Brain Topography*. 2013. 26: 428-441.

Lemon RN. The output map of the primate motor cortex. *Trends of Neuroscience*. 1988. 11: 501-506.

Levelt WJ1, Roelofs A, Meyer AS. A theory of lexical access in speech production. *Behavioral and Brain Sciences*. 1999. 22: 1-38; discussion 38-75.

Liberman AM, Cooper FS, Shankweiler DP, Studdert-Kennedy M. Perception of the speech code. *Psychological Review*. 1967. 74: 431-461.

Liberman AM, Mattingly IG. The motor theory of speech perception revised. *Cognition*. 1985. 21: 1-36.

Liljeström M, Stevenson C, Kujala J, Salmelin R. Task- and stimulus-related cortical networks in language production: Exploring similarity of MEG- and fMRI-derived functional connectivity. *Neuroimage*. 2015. 120: 75-87.

Liscic RM, Zidar J, Mihelin M. Evidence of direct connection of corticobulbar fibers to orofacial muscles in man: electromyographic study of individual motor unit responses. *Muscle Nerve*. 1998. 21: 561-566.

Livingston SC, Goodkin HP, Ingersoll CD. The influence of gender, hand dominance, and upper extremity length on motor evoked potentials. *Journal of Clinical Monitoring and Computing*. 2010. 24: 427-436

Long MA, Katlowitz KA, Svirsky MA, Clary RC, Byun TM, Majaj N, Oya H, Howard MA, Greenlee JD. Functional Segregation of Cortical Regions Underlying Speech Timing and Articulation. *Neuron*. 2016. 89: 1187-1193.

Lüders H, Lesser RP, Morris HH, Dinner DS, Hahn J. Negative motor responses elicited by stimulation of the human cortex. *Advances in Epileptology*. 1987. 16: 229-231.

Lüders HO. The supplementary sensorimotor area. An overview. *Advances in Neurology*. 1996. 70: 1-16.

Luppino G, Matelli M, Camarda RM, Gallese V, Rizzolatti G. Multiple representations of body movements in mesial area 6 and the adjacent cingulate cortex: an intracortical microstimulation study in the macaque monkey. *Journal of Comparative Neurology*. 1991. 311: 463-482.

Maas E, Robin DA, Wright DL, Ballard KJ. Motor programming in apraxia of speech. *Brain and Language*. 2008. 106: 107-118.

Maier M, Armand J, Kirkwood PA, Yang HW, Davis JN, Lemon RN. Differences in the corticospinal projection from primary motor cortex and supplementary motor area to macaque upper limb motoneurons: an anatomical and electrophysiological study. *Cerebral Cortex*. 2002. 12: 281-296.

Maier MA, Kirkwood PA, Brochier T, Lemon RN. Responses of single corticospinal neurons to intracortical stimulation of primary motor and premotor cortex in the anesthetized macaque monkey. *Journal of Neurophysiology*. 2013. 109: 2982-2998.

Mandonnet E, Sarubbo S, Duffau H. Proposal of an optimized strategy for intraoperative testing of speech and language during awake mapping. *Neurosurgery Review*. 2016. [Epub ahead of print]

Makris N, Zhu A, Papadimitriou GM, Mouradian P, Scaccianoce E, Baselli G, Baglio F, Shenton ME, Rathi Y, Dickerson B, Yeterian E, Kubicki M. Mapping temporo-parietal and temporo-occipital cortico-cortical connections of the human middle longitudinal fascicle in subject-specific, probabilistic, and stereotaxic Talairach spaces. *Brain Imaging and Behavior*. 2016. [Epub ahead of print]

Maranesi M, Rodà F, Bonini L, Rozzi S, Ferrari PF, Fogassi L, Coudé G. Anatomo-functional organization of the ventral primary motor and premotor cortex in the macaque monkey. *European Journal of Neuroscience*. 2012. 36: 3376-3387.

Markram H, Wang Y, Tsodyks M. Differential signaling via the same axon of neocortical pyramidal neurons. *Proceding National Academy of Science*. 1998. 95: 5323-5328.

Matsuda R, Moritz-Gasser S, Duvaux S, Fernández Coello A, Martinoni M, Duffau H. The persistent crucial role of the left hemisphere for language in left-handers with a left low grade glioma: a stimulation mapping study. *Acta Neurochirurgica (Wien)*. 2014. 156: 661-670.

McGuinness E, Sivertsen D, Allman JM. Organization of the face representation in macaque motor cortex. *Journal of comparative neurology*. 1980. 193: 591-608.

Meyer BU, Werhahn K, Rothwell JC, Roericht S, Fauth C. Functional organisation of corticonuclear pathways to motoneurones of lower facial muscles in man. *Experimental Brain Research*. 1994. 101: 465-472.

Milner AD, Goodale MA. Visual pathways to perception and action. *Progress in Brain Research*. 1993. 95 :317-337.

Mink JW. The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in Neurobiology*. 1996. 50: 381–425.

Mishkin M, Lewis ME, Ungerleider LG. Equivalence of parieto-preoccipital subareas for visuospatial ability in monkeys. *Behavioral and Brain Research*. 1982. 6:41-55.

Mohr JP, Pessin MS, Finkelstein S, Funkenstein HH, Duncan GW, Davis KR. Broca aphasia: pathologic and clinical. *Neurology*. 1978. 28, 311-324.

Moore CJ, Price CJ. A functional neuroimaging study of the variables that generate category-specific object processing differences. *Brain*. 1999. 122: 943-962.

Moorman S, Gobes SM, Kuijpers M, Kerkhofs A, Zandbergen MA, Bolhuis JJ. Human-like brain hemispheric dominance in birdsong learning. *Proceedings of the national academy of sciences*. 2012. 109:12782-12787.

Morecraft RJ, Louie JL, Herrick JL, Stilwell-Morecraft KS. Cortical innervation of the facial nucleus in the non-human primate: a new interpretation of the effects of stroke and related subtotal brain trauma on the muscles of facial expression. *Brain*. 2001. 124: 176-208.

Morecraft RJ, Stilwell-Morecraft KS, Rossing WR. The motor cortex and facial expression: new insights from neuroscience. *Neurologist*. 2004. 10: 235-249.

Morecraft RJ, Ge J, Stilwell-Morecraft K, McNeal D, Pizzimenti MA, Darling WG. Terminal distribution of the corticospinal projection from the hand/arm region of the primary motor cortex to the cervical enlargement in rhesus monkey. *Journal of Comparative Neurology*. 2013. 521: 4205-4235.

Moro A, Tettamanti M, Perani D, Donati C, Cappa SF, Fazio F. Syntax and the brain: disentangling grammar by selective anomalies. *Neuroimage*. 2001. 13: 110-118.

Musso M, Moro A, Glauche V, Rijntjes M, Reichenbach J, Büchel C, Weiller C. Broca's area and the language instinct. *Nature Neuroscience*. 2003. 6: 774-781.

Nachev P, Kennard C, Husain M. Functional role of the supplementary and pre-supplementary motor areas. *Nature Reviews Neuroscience*. 2008. 9: 856-869.

New AB, Robin DA, Parkinson AL, Duffy JR, Mcneil MR, Piguet O, Ballard KJ. Clinical Altered resting-state network connectivity in stroke patients with and without apraxia of speech. *Neuroimage: clinical*. 2015. 8: 429-439.

Oh A, Duerden EG, Pang EW. The role of the insula in speech and language processing. *Brain and Language*. 2014. 135: 96-103.

Ojemann G, Ojemann J, Lettich E, Berger M. Cortical language localization in left, dominant hemisphere. An electrical stimulation mapping investigation in 117 patients. *Journal of Neurosurgery*. 1989. 71: 316-326.

Oller DK, Eilers RE. The role of audition in infant babbling. *Child Development*. 1988. 59: 441-449.

Olson IR, Von Der Heide RJ, Alm KH, Vyas G. Development of the uncinate fasciculus: Implications for theory and developmental disorders. *Developmental Cognitive Neuroscience*. 2015. 14: 50-61.

Orgogozo JM, Larsen B. Activation of the supplementary motor area during voluntary movement in man suggests it works as a supramotor area. *Science*. 1979. 206: 847-850.

Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. *Journal of Physiology*. 2008. 586: 5147-5159..

Papagno C, Miracapillo C, Casarotti A, Romero Lauro LJ, Castellano A, Falini A, Casaceli G, Fava E, Bello L. What is the role of the uncinate fasciculus? Surgical removal and proper name retrieval. *Brain*. 2011. 134: 405-414.

Papoutsi M, de Zwart JA, Jansma JM, Pickering MJ, Bednar JA, Horwitz B. From phonemes to articulatory codes: an fMRI study of the role of Broca's area in speech production. *Cerebral Cortex*. 2009. 19: 2156-2165.

Paradiso GO, Cunic DI, Gunraj CA, Chen R. Representation of facial muscles in human motor cortex. Journal of Physiology. 2005. 567: 323-336.

Patton HD, Amassian V. Single and multiple-unit analysis of cortical stage of pyramidal tract activation. *Journal of Neurophysiology*. 1954. 17: 345-363.

Peeva MG, Guenther FH, Tourville JA, Nieto-Castanon A, Anton JL, Nazarian B, Alario FX. Distinct representations of phonemes, syllables, and supra-syllabic sequences in the speech production network. *Neuroimage*. 2010. 50: 626-638.

Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain*. 1937. 9: 389-443.

Penfield W, Welch K. The supplementary motor area of the cerebral cortex; a clinical and experimental study. *A.M.A Archives of neurology and psychiatry*. 1951. 66: 289-317.

Petrovich N, Holodny AI, Tabar V, Correa DD, Hirsch J, Gutin PH, Brennan CW. Discordance between functional magnetic resonance imaging during silent speech tasks and intraoperative speech arrest. *Journal of Neurosurgery*. 2005. 103: 267-274.

Pfenning AR, Hara E, Whitney O, Rivas MV, Wang R, Roulhac PL, Howard JT, Wirthlin M, Lovell PV, Ganapathy G, Mouncastle J, Moseley MA, Thompson JW, Soderblom EJ, Iriki A, Kato M, Gilbert MT, Zhang G, Bakken T, Bongaarts A, Bernard A, Lein E, Mello CV, Hartemink AJ, Jarvis ED. Convergent transcriptional specializations in the brains of humans and song-learning birds. *Science*. 2014. 346:1256846. doi: 10.1126/science.1256846.

Pilurzi G, Hasan A, Saifee TA, Tolu E, Rothwell JC, Deriu F. Intracortical circuits, sensorimotor integration and plasticity in human motor cortical projections to muscles of the lower face. *Journal of Physiology*. 2013. 591: 1889-1906.

Pinheiro JC, Douglas MB. Mixed-effects models in S and S-PLUS. Springer, New York, 2000.

Porter R, Lemon RN. Corticospinal function and voluntary movement. Oxford: Oxford University Press. 1993.

Prabhu G, Shimazu H, Cerri G, Brochier T, Spinks RL, Maier MA, Lemon RN. Modulation of primary motor cortex outputs from ventral premotor cortex during visually guided grasp in the macaque monkey. *Journal of Neurophysiolology*. 2009. 587:1057-1069.

Preuss TM, Stepniewska I, Kaas JH. Movement representation in the dorsal and ventral premotor areas of owl monkeys: a microstimulation study. *Journal of Comparative Neurology*. 1996. 371: 649-676.

Price CJ. The anatomy of language: a review of 100 fMRI studies published in 2009. *Annals of the New York Academy of Sciences*. 2010. 1191: 62-88.

Raos V, Umiltà MA, Gallese V, Fogassi L. Functional properties of grasping-related neurons in the dorsal premotor area F2 of macaque monkey. *Journal of Neurophysiology*. 2004. 92: 1990-2002.

Raos V, Umilta´ MA, Murata A, Fogassi L, Gallese V. Functional properties of grasping-related neurons in the ventral premotor area f5 of the macaque monkey. *Journal of Neurophysiology*. 2006. 95: 709-729.

Rathelot JA, Strick PL. Muscle representation in the macaque motor cortex: an anatomical perspective. *Proceedings of the national academy of sciences*. 2006. 103: 8257-8262.

Rathelot JA, Strick PL. Subdivisions of primary motor cortex based on cortico-motoneuronal cells. *Proceedings of the national academy of sciences*. 2009. 106: 918-923.

Redford MA. The Handbook of Speech Production, First Edition. John Wiley & Sons, Inc. Published 2015.

Riede T, Goller F. Peripheral mechanisms for vocal production in birds - differences and similarities to human speech and singing. *Brain and Language*. 2010. 115: 69-80.

Riva M, Fava E, Gallucci M, Comi A, Casarotti A, Alfiero T, Raneri FA, Pessina F, Bello L. Monopolar high-frequency language mapping: can it help in the surgical management of gliomas? A comparative clinical study. *Journal of Neurosurgery*. 2016. 124: 1479-1489.

Rizzolatti G, Camarda R, Fogassi L, Gentilucci M, Luppino G, Matelli M. Functional organization of inferior area 6 in the macaque monkey. II. Area F5 and the control of distal movements. *Experimental Brain Research*. 1988. 71: 491-507.

Rizzolatti G, Fadiga L, Matelli M, Bettinardi V, Paulesu E, Perani D, Fazio F. Localization of grasp representations in humans by PET: 1. Observation versus execution. *Experimental Brain Research*. 1996. 111: 246-252.

Rizzolatti G, Fogassi L, Gallese V. Parietal cortex: from sight to action. Current Opinion in Neurobiology. 1997. 7: 562-567.

Rizzolatti G, Cattaneo L, Fabbri-Destro M, Rozzi S. Cortical mechanisms underlying the organization of goal-directed actions and mirror neuron-based action understanding. *Physiology Review*. 2014. 94: 655-706.

Robinson G, Shallice T, Bozzali M, Cipolotti L. The differing roles of the frontal cortex in fluency tests. *Brain*. 2012. 135: 2202-2214.

Rödel R, Laskawi R, Markus H. Transcranial cortical magnetic stimulation of lower-lip mimetic muscles: effect of coil position on motor evoked potentials. *Journal for oto-rhino-laryngology and its related specialties*. 1999. 61: 119-125.

Routal RV, Pal GP. Location of the phrenic nucleus in the human spinal cord. *Journal of Anatomy*. 1999. 195: 617-621.

Ruge M, Victor J, Hosain S, Correa DD, Relkin NR, Tabar V, Brennan C, Gutin PH, Hirsch J. Concordance between functional magnetic resonance imaging and intraoperative language mapping. *Stereotactic and Functional Neurosurgery*. 1999. 72: 95-102.

Salmelin R, Helenius P, Service E. Neurophysiology of fluent and impaired reading: a magnetoencephalographic approach. *Journal of Clinical Neurophysiology*. 2000. 17: 163-174.

Sanai N, Mirzadeh Z, Berger MS. Functional outcome after language mapping for glioma resection. *The New England Journal of Medicine*. 2008. 358: 18-27.

Sanes JN, Donoghue JP, Thangaraj V, Edelman RR, Warach S. Shared neural substrates controlling hand movements in human motor cortex. *Science*. 1995. 268: 1775-1777.

Sarubbo S, De Benedictis A, Merler S, Mandonnet E, Barbareschi M, Dallabona M, Chioffi F, Duffau H. Structural and functional integration between dorsal and ventral language streams as revealed by blunt dissection and direct electrical stimulation. *Human Brain Mapping*. 2016. 37: 3858-3872.

Schäffler L, Lüders HO, Dinner DS, Lesser RP, Chelune GJ. Comprehension deficits elicited by electrical stimulation of Broca's area. *Brain*. 1993. 116: 695-715.

Schieber MH. Individuated finger movements of rhesus monkeys: a means of quantifying the independence of the digits. *Journal of Neurophysiology*. 1991. 65: 1381-1391.

Schieber MH. Constraints on somatotopic organization in the primary motor cortex. *Journal of Neurophysiology*. 2001. 86: 2125-2143.

Schmidlin E, Brochier T, Maier MA, Kirkwood PA, Lemon RN. Pronounced reduction of digit motor responses evoked from macaque ventral premotor cortex after reversible inactivation of the primary motor cortex hand area. *The Journal of Neuroscience*. 2008. 28: 5772-5783.

Schmitt BM, Münte TF, Kutas M. Electrophysiological estimates of the time course of semantic and phonological encoding during implicit picture naming. *Psychophysiology*. 2000. 37: 473-484.

Schmitt BM1, Schiltz K, Zaake W, Kutas M, Münte TF. An electrophysiological analysis of the time course of conceptual and syntactic encoding during tacit picture naming. *Journal of Cognitive Neuroscience*. 2001. 13: 510-522.

Schulz GM, Varga M, Jeffires K, Ludlow CL, Braun AR. Functional neuroanatomy of human vocalization: an H215O PET study. *Cerebral Cortex*. 2005. 15: 1835-1847.

Scott CM, Ringel RL. The effects of motor and sensory disruptions on speech: a description of articulation. *Journal of Speech language and Hearing Research*. 1971. 14: 819-828.

Sessle BJ, Wiesendanger M. Structural and functional definition of the motor cortex in the monkey (Macaca fascicularis). *Journal of physiology*. 1982. 323: 245-265.

Shattuck DW, Leahy RM. BrainSuite: an automated cortical surface identification tool. *Medical Image Analysis*. 2002. 6: 129-142.

Sherwood CC, Holloway RL, Erwin JM, Hof PR. Cortical orofacial motor representation in Old World monkeys, great apes, and humans. II. Stereologic analysis of chemoarchitecture. *Brain, Behavior and Evolution*. 2004a. 63: 82-106.

Sherwood CC, Holloway RL, Erwin JM, Schleicher A, Zilles K, Hof PR. Cortical orofacial motor representation in Old World monkeys, great apes, and humans. I. Quantitative analysis of cytoarchitecture. *Brain, Behavior and Evolution*. 2004b. 63: 61-81.

Sherwood CC, Hof PR, Holloway RL, Semendeferi K, Gannon PJ, Frahm HD, Zilles K. Evolution of the brainstem orofacial motor system in primates: a comparative study of trigeminal, facial, and hypoglossal nuclei. *Journal of Human Evolution*. 2005. 48: 45-84.

Shimazu H, Maier MA, Cerri G, Kirkwood PA, Lemon RN. Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. *Journal of Neuroscience*. 2004. 24: 1200-1211.

Showers MJ, Lauer EW. Somatovisceral motor patterns in the insula. *Journal of Comparative Neurology*. 1961. 117: 107-115.

Silberberg G, Markram H. Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron*. 2007. 53: 735-746.

Simonyan K, Jürgens U. Efferent subcortical projections of the laryngeal motorcortex in the rhesus monkey. *Brain Research*. 2003. 974: 43-59.

Simonyan K, Jürgens U. Afferent subcortical connections into the motor cortical larynx area in the rhesus monkey. *Neuroscience*. 2005. 130: 119-131.

Simonyan K, Horwitz B. Laryngeal motor cortex and control of speech in humans. *Neuroscientist*. 2011. 17: 197-208.

Skeide MA, Brauer J, Friederici AD. Brain Functional and Structural Predictors of Language Performance. *Cerebral Cortex*. 2016. 26: 2127-2139.

Smith CR. Interjected sounds in deaf children's speech. *Journal of Communication Disorders*. 1975. 8: 123-128.

Sohn YH, Hallett M. Surround inhibition in human motor system. *Experimental Brain Research*. 2004. 158: 397-404.

Szelényi A, Bello L, Duffau H, Fava E, Feigl GC, Galanda M, Neuloh G, Signorelli F, Sala F. Intraoperative electrical stimulation in awake craniotomy: methodological aspects of current practice. *Neurosurgery Focus*. 2010. 28: E7.

Tadel F, Baillet S, Mosher JC, Pantazis D, Leahy RM. Brainstorm: a user-friendly application for MEG/EEG analysis. *Computational Intelligence and Neuroscience*. 2011. 2011:879716. doi: 10.1155/2011/879716.

Taniguchi M, Cedzich C, Schramm J. Modification of cortical stimulation for motor evoked potentials under general anesthesia: technical description. *Neurosurgery*. 1993. 32: 219-226.

Tate MC, Herbet G, Moritz-Gasser S, Tate JE, Duffau H. Probabilistic map of critical functional regions of the human cerebral cortex: Broca's area revisited. *Brain*. 2014. 137: 2773-2782.

Tate MC, Herbet G, Moritz-Gasser S, Tate JE, Duffau H. Reply: probabilistic map of language regions: challenge and implication. *Brain*. 2015. 138: e338.

Tattersall I. Human evolution and cognition. *Theory in Biosciences*. 2010. 129:193-201.

Thorpe S, Fize D, Marlot C. Speed of processing in the human visual system. *Nature*. 1996. 381: 520-522.

Triggs WJ, Ghacibeh G, Springer U, Bowers D. Lateralized asymmetry of facial motor evoked potentials. *Neurology*. 2005. 65: 541-544.

Tokuno H, Takada M, Nambu A, Inase M. Reevaluation of ipsilateral corticocortical inputs to the orofacial region of the primary motor cortex in the macaque monkey. *Journal of Comparative Neurology*. 1997. 389: 34-48.

Tomasch J. The numerical capacity of the pontine cell and fibre systems. *Journal of Anatomy*. 1969. 104: 187.

Tomasino B, Marin D, Canderan C, Maieron M, Budai R, Fabbro F, Skrap M. Involuntary switching into the native language induced by electrocortical stimulation of the superior temporal gyrus: a multimodal mapping study. *Neuropsychologia*. 2014. 62 :87-100.

Trojaborg W. Does cross-innervation occur after facial palsy? *Journal of Neurology, Neurosurgery & Psychiatry*. 1977. 40: 712-717.

Umiltà MA, Brochier T, Spinks RL, Lemon RN. Simultaneous recording of macaque premotor and primary motor cortex neuronal populations reveals different functional contributions to visuomotor grasp. *Journal of Neurophysiology*. 2007. 98: 488–501.

Umiltà MA, Escola L, Intskirveli I, Grammont F, Rochat M, Caruana F, Jezzini A, Gallese V, Rizzolatti G. When pliers become fingers in the monkey motor system. *Proceedings of the national academy of sciences*. 2008. 105: 2209-2213.

Urban PP, Beer S, Hopf HC. Cortico-bulbar fibers to orofacial muscles: recordings with enoral surface electrodes. *Electroencephalography and Clinical Neurophysiology*. 1997. 105:8-14.

Urban PP, Wicht S, Hopf HC. Sensitivity of transcranial magnetic stimulation of cortico-bulbar vs. cortico-spinal tract involvement in Amyotrophic Lateral Sclerosis (ALS). *Journal of Neurology*. 2001. 248: 850-855.

van Turennout M, Hagoort P, Brown CM. Electrophysiological evidence on the time course of semantic and phonological processes in speech production. *Journal of Experimental Psychology: Learning, Memory and Cognition*. 1997. 23: 787-806.

van Turennout M, Hagoort P, Brown C. The time course of grammatical and phonological processing during speaking: evidence from event-related brain potentials. *Journal of Psycholinguist Research*. 1999. 28: 649-676.

van Turennout M, Ellmore T, Martin A. Long-lasting cortical plasticity in the object naming system. *Nature Neuroscience*. 2000. 3: 1329-1334.

Vergani F, Lacerda L, Martino J, Attems J, Morris C, Mitchell P, Thiebaut de Schotten M, Dell'Acqua F. White matter connections of the supplementary motor area in humans. *Journal of neurology, neurosurgery and psychiatry*. 2014. 85: 1377-1385.

Verstynen T, Jarbo E, Pathak S, Schneider W. In Vivo mapping of microstructural somatotopies in the human corticospinal pathways. *Journal of Neurophysiology*. 2011. 105:336–346.

Vigneau M, Beaucousin V, Hervé PY, Duffau H, Crivello F, Houdé O, Mazoyer B, Tzourio-Mazoyer N. Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. *Neuroimage*. 2006. 30: 1414-1432.

Vigneswaran G, Kraskov A, Lemon RN. Large identified pyramidal cells in macaque motor and premotor cortex exhibit "thin spikes": implications for cell type classification. *Journal of Neuroscience*. 2011. 31: 14235-14242.

Wise SP, Boussaud D, Johnson PB, Caminiti R. Premotor and parietal cortex: corticocortical connectivity and combinatorial computation. *Annuals Review Neuroscience*. 1997. 20: 25-42.

Wise RJ, Greene J, Büchel C, Scott SK. Brain regions involved in articulation. *Lancet*. 1999. 353: 1057-1061.

Yildiz N, Yildiz S, Ertekin C, Aydoğdu I, Uludag B. Changes in the perioral muscle responses to cortical TMS induced by decrease of sensory input and electrical stimulation to lower facial region. *Clinical Neurophysiology*. 2004. 115: 2343-2349.

Zhang GX, Sasamoto K. Projections of two separate cortical areas for rhythmical jaw movements in the rat. *Brain Research Bulletin*. 1990. 24: 221-230.

Ziegler W, Aichert I. How much is a word? Predicting ease of articulation planning from apraxic speech error patterns. *Cortex*. 2015. 69: 24-39.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *Journal of Physiology*. 1996. 496: 873-881.