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QUANTITATIVE ANALYSIS OF THE SPEECH AND LIP MOVEMENTS THROUGH OPTOELECTRONIC MOTION ANALYSIS AND SURFACE ELECTROMYOGRAPHY

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I dedicate this work to my parents who raised me and always supported me in my decisions.

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2 LIST OF ABBREVIATIONS

2D: bi-dimensional

3D: three-dimensional

ANOVA: analysis of variance

ASYM: asymmetry index for sequence of natural numbers and words

CCD: charge coupled device

EMG: electromyography

NS: not significant

OMES - Orofacial Myofunctional Evaluation with Scores Protocol

RDC: research diagnostic criteria

RMS: root mean square

SD: standard deviation

TMD: temporomandibular disorder

TMJ: temporomandibular joint

3 ABSTRACT

Functional impairments of facial movements alter the quality of life, and their quantitative analysis is a key step in the description and grading of facial function and dysfunction. In this investigation we assessed the symmetry of lip movements in verbal and non-verbal movements in healthy subjects.

A non-invasive recording protocol, integrating an electromyographic system and an optoelectronic 3D-motion analyzer, has been developed and used to detect lip movements in verbal and non-verbal movements.

Two separate investigations have been made. In the first study, functional symmetries of the lip movements were assessed in a control group of clinically healthy subjects. Data were evaluated separately for men and women, and a gender-related effect was tested.

The aim of the second study was to assess the onset of the EMG activity of zygomaticus and depressor labii inferioris muscles that play a role in speech pronunciation and smiling movements.

The outcomes suggest that the proposed method could be a useful tool to evaluate the asymmetry of the lips and of the facial muscles during the performance of smiling, lip purse and speech pronunciation, and to detect functionally altered facial conditions.

Key words: 3D motion analysis; electromyography; health; speech; facial muscles.

4 INTRODUCTION

The face characterizes human beings and in particular the mouth and lips plays a key role in the evaluation and recognition of the craniofacial complex.

The smile is one of the most frequent facial expressions, and is used to transmit positive emotional state, as well as to serve social functions such as greeting. Like many facial expressions, the smile can be produced either deliberately by voluntary movement of the Zygomaticus major muscles or spontaneously (Lapatki et al., 2003; Schmidt et al., 2006).

During speech pronunciation, the movements of the articulators create temporal sequences of sounds, characterized by activations of the tongue, jaw, lips, vocal folds and velum muscles, i.e. movements of the vocal tract articulators, and the resulting vocal tract shapes correspond to the produced patterns of speech. At the extrinsic level, acoustic signals, visual and perceptual salience similarly correspond to the produced patterns of speech (Smith 1992; Green et al., 2000; Bianchini and Andrade, 2006; Van der Geld et al., 2008; Sawyer et al., 2010; Grimme et al., 2011).

For speech pronunciation the timing is critical, since it can carry relevant information for the communication process, and generally involves the coordination of different end-effectors and different movements of the face, and requires a sequence of well-coordinated orofacial movements (Grimme et al., 2011).

Facial expressions can be altered in various pathologic conditions and malformations, deriving from central nervous system diseases, neuromuscular and peripheral nerve paralysis (mostly, facial nerve paralysis), drug administration, dentofacial deformities and scars, congenital anomalies (Trotman et al., 1998a; Okada, 2001; Wachtman et al., 2001; Mishima et al., 2004; Nooreyazdan et al., 2004; Tarantili et al., 2005; Tzou et al., 2005; Proff et al., 2006; Agostino et al., 2008; Mehta et al., 2008; Sawyer et al., 2010; Sforza et al., 2012).

In several medical and dental fields, facial dysfunctions are usually assessed independently from their origin, and several clinical and instrumental assessments can be used to grade both spontaneous and instructed movements (Trotman et al., 2000; Wachtman et al., 2001; Linstrom et al., 2002; Giovanoli et al., 2003; Nooreyazdan et al., 2004; Proff et al., 2006; Ferrario and Sforza, 2007; Hontanilla and Aubá, 2008; Mehta et al., 2008; Popat et al., 2008a).

Clinical assessments focalize on total and local facial motion, synkinesis and movement asymmetries (Proff et al., 2006; Ferrario and Sforza, 2007; Reitzen et al., 2009), whilst quantitative methods can assess both the movements of selected facial landmarks and their trajectories (Linstrom et al., 2002; Giovanoli et al., 2003; Ferrario and Sforza, 2007; Hontanilla and Aubá, 2008; Mehta et al., 2008; Popat et al., 2008b; Sawyer et al., 2010). Clinical evaluations can have a reduced inter-examiners repeatability, with problems in data sharing among different care takers or research centers. To overcome these limitations, quantitative methods for the assessment of facial movements have been proposed (Trotman et al., 2000; Okada, 2001; Kang et al., 2002; Linstrom et al., 2002; Tzou et al., 2005; Proff et al., 2006).

Nowadays, several three-dimensional motion analyzers allow a non-invasive quantitative assessment of soft tissue facial movements without interfering with the subject (Weeden et al., 2001; Coulson et al., 2000; 2002; Giovanoli et al., 2003; Johnston et al., 2003; Mishima et al., 2004; Nooreyazdan et al., 2004; Proff et al., 2006; Ferrario and Sforza, 2007; Agostino et al., 2008; Popat et al., 2008a,b; Sforza et al., 2010b,c; 2012; Verzé et al., 2011a,b).

The detection and quantitative analysis of facial movements is a key step in the description and grading of facial function and dysfunction, during diagnosis, treatment and follow-up of their disorders (Trotman et al., 2000; Okada et al., 2001; Coulson et al., 2002; Kang et al., 2002; Tzou et al., 2005; Proff et al., 2006; Hontanilla and Aubá, 2008; Mehta et al., 2008; Okamoto et al., 2010; Sawyer et al., 2010).

In our laboratory, we developed a method for the non-invasive, three-dimensional assessment of facial movements using an optoelectronic motion analyser (Ferrario and Sforza, 2007; Sforza et al., 2010b,c; 2012). This method was found to be minimally disturbing, reliable, and to accurately detect total and local motion during the performance of standardized facial animations (Sforza et al., 2010b,c; 2012). It offers a valuable support for the extraction of numeric values, which are very useful in the differential diagnosis (Ferrario and Sforza, 2007; Popat et al., 2008a,b; Mapelli et al., 2009; Sforza et al., 2010b; Verzé et al., 2011a,b).

Patient can be compared to reference values obtained from healthy individuals according to age, gender and ethnicity (Frey et al., 1999; Tzou et al., 2005; Sforza et al., 2010b,c). Longitudinal assessments can also be performed during treatment and rehabilitation.

Another useful system in the differential diagnosis process and in the therapeutic planning is surface electromyography (EMG). EMG can monitor orofacial functions, it is highly sensitive in measuring muscle performance, monitoring their efficiency, and comparing different groups of individuals (Ferrario et al., 2000a, 2006a,b, Galo et al., 2006, 2007; Tartaglia et al 2008; Felício et al., 2009b, 2012).

Among the various assessments of facial function, the detection of symmetry (or its counterpart, asymmetry) is one of the most important, and even lay observers can detect facial asymmetries at rest and during mimicry. Considering the well-known fluctuating asymmetry existing in all subjects (Sforza et al., 2010d), before defining clinical values for "asymmetry", it is necessary to define a threshold in healthy individuals.

Several studies about the quantitative evaluation of the symmetric and asymmetric facial movements using non-invasive methods like three-dimensional optoelectronic analysis (Ferrario and Sforza, 2007; Sforza et al. 2010 b,c; 2012) and EMG (Ferrario et al., 2000a, 2001; Felício et al., 2009b) were performed, but in all cases only not-verbal animations were analyzed. Indeed, facial and labial movements during speech production are an integral part of the social life of each person, and their quantitative analysis is mandatory in several clinical fields.

The assessments of healthy individuals would provide data that can be used as normality parameters for the comparison with the patients with morphologic and/or functional problems in the skull-facial area.

In the present investigation, three-dimensional motion analysis and surface EMG have been applied to analyse both facial kinematics and facial muscle function in a group of young healthy subjects.

5 ANATOMY AND FUNCTION

5.1 FACE

The aspect of the face, from above downwards, consists of the temporal region, cheek and lower jaw. The temporal region lies in front of the external ear and above the zygomatic arch. It is demarcated superiorly by the superior and inferior temporal lines, and inferiorly and laterally by the zygomatic arch.

The variable prominence of the zygoma is largely attributable to the shape of the body of the underlying zygomatic bone. If the sharp posterior margin of the frontal process of the zygomatic bone is followed upwards, its fusion with the zygomatic process of the frontal bone at the zygomaticofrontal suture may be detected.

The face should be divided into three parts (Fig.1). The upper third of the face is outlined superiorly by hairline and inferiorly by the glabella and frontonasal groove (centrally), and laterally by the eyebrows and the supraorbital ridges.

The middle third of the face is defined as that area bounded above by a transverse line connecting the two zygomaticofrontal sutures, passing through the frontomaxillary and frontonasal sutures, and limited below by the occlusal plane of the maxillary teeth. Posteriorly the region is limited by the sphenoethmoidal junction, but it includes the free margins of the pterygoid plates inferiorly.

The lower third of face extends from subnasale to menton. The lower third is further divided into its own thirds, with the upper lip occupying one third, and the lower lip and chin defined occupying the other two thirds.

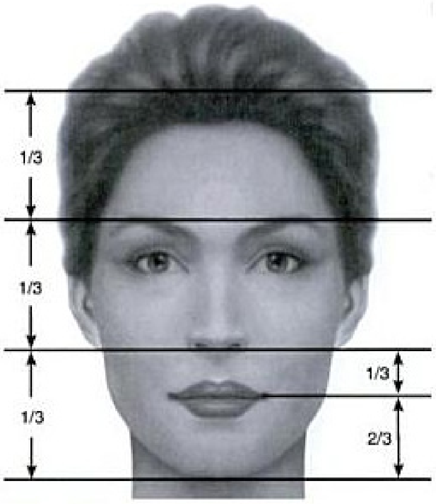


Figure 1. Thirds of facial proportions (Papel et al., 2008).

5.2 FACIAL NERVE

The facial nerve emerges from the skull base at the stylomastoid foramen and almost immediately gives off the nerves to the posterior belly of digastric and stylohyoid, and the posterior auricular nerve, which supplies the occipital belly of occipitofrontalis and some of the auricular muscles (Fig. 2).

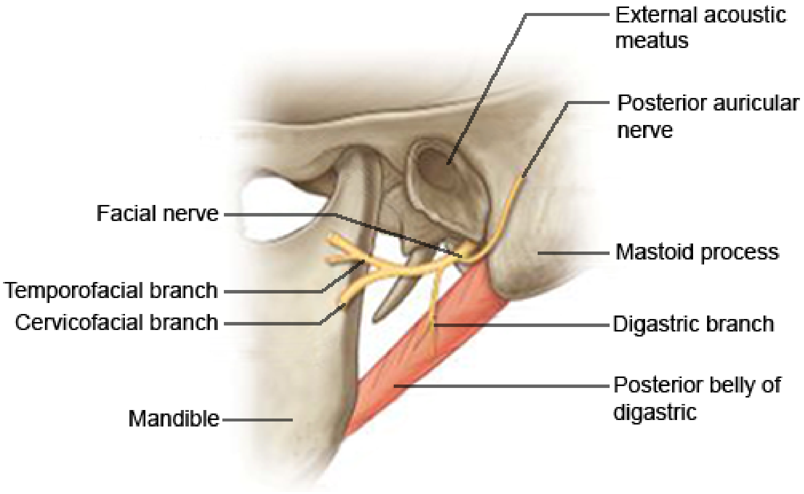


Figure 2. Distribution of the facial nerve. The branches given off immediately after the nerve exits the stylomastoid foramen. (Standring et al., 2008, modified).

The nerve next enters the parotid gland high up on its posteromedial surface and passes forwards and downwards behind the mandibular ramus. Within the substance of the gland it branches into superior (temporofacial) and inferior (cervicofacial) trunks, usually just behind and superficial to the retromandibular vein. The trunks branch further to form a parotid plexus. Five main terminal branches arise from the plexus, they diverge within the gland and leave by its anteromedial surface, medial to its anterior margin, to supply the muscles of facial expression (Fig. 3).

Figure 3. The branches of the facial nerve on the face. (Rizzolo and Madeira, 2005, modified)

The temporal branch usually divides into anterior and posterior rami soon after piercing the parotidomasseteric fascia below the zygomatic arch; there is often a middle (frontal) ramus. Twigs supply intrinsic muscles on the lateral surface of the auricle, and the anterior and superior auricular muscles, and communicate with the zygomaticotemporal branch of the maxillary nerve and the auriculotemporal branch of the mandibular nerve. The more anterior branches supply the frontal belly of occipitofrontalis, orbicularis oculi and corrugator, and join the supraorbital and lacrimal branches of the ophthalmic nerve.

Zygomatic branches are generally multiple. They cross the zygomatic bone to the lateral canthus of the eye and supply orbicularis oculi: they may also supply muscles innervated by the buccal branch. Twigs communicate with filaments of the lacrimal nerve and the zygomaticofacial branch of the maxillary nerve.

The buccal branch is usually single. It has a close relationship to the parotid duct for about 2.5 cm after emerging from the parotid gland, and typically lies below the duct. Superficial branches run beneath the subcutaneous fat and superficial musculo-aponeurotic system. Some branches pass deep to procerus and join the infratrochlear and external nasal nerves. Upper deep branches supply zygomaticus major and levator labii superioris, and form an infraorbital plexus with the superior labial branches of the infraorbital nerve. They also supply levator anguli oris, zygomaticus minor, levator labii superioris alaequae nasi and the small nasal muscles: these branches are sometimes described as lower zygomatic branches. Lower deep branches supply buccinator and orbicularis oris; they communicate with filaments of the buccal branch of the mandibular nerve.

There are usually two marginal mandibular branches. They run forwards towards the angle of the mandible under platysma, then turn upwards across the body of the mandible to pass under depressor anguli oris. The branches supply risorius and the muscles of the lower lip and chin, and filaments communicate with the mental nerve.

The cervical branch emerges from the lower part of the parotid gland and runs anteroinferiorly under platysma to the front of the neck. Typically single, it supplies platysma and communicates with the transverse cutaneous cervical nerve.

Cutaneous branches of the facial nerve accompany the auricular branch of the vagus; they are believed to innervate the skin on both auricular aspects, in the conchal depression and over its eminence.

5.3 MUSCLES OF FACIAL EXPRESSION

The shape of the mouth and labial postures are controlled by an intricate three-dimensional complex of muscular slips, including: the elevators, retractors and evertors of the upper lip and buccal angle (levator labii superioris alaeque nasi, levator labii superioris, zygomaticus major and minor, levator anguli oris and risorius); the depressors, retractors and evertors of the lower lip and buccal angle (depressor labii inferioris, depressor anguli oris and mentalis); antagonists of the foregoing, a compound sphincter (orbicularis oris with its sub regions, incisivus superior and inferior) and buccinator.

Although these muscles produce movements of the facial skin that reflect emotions, it is usually argued that their primary function is to act as sphincters and dilators of the facial orifices and that the function of facial expression has developed secondarily. Embryologically, they are derived from the mesenchyme of the second pharyngeal arch and so are innervated by the facial nerve. Topographically and functionally the muscles of facial expression may be subdivided into epicranial, circumorbital and palpebral, nasal, and buccolabial groups (Fig. 4).

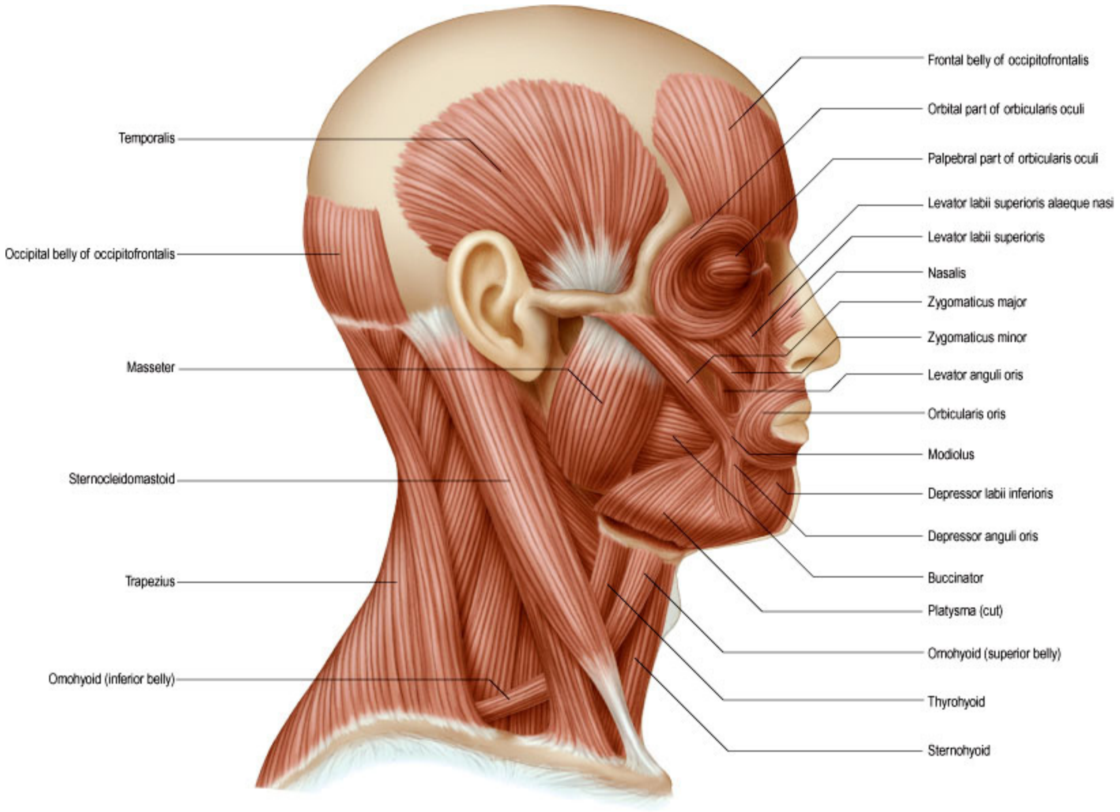


Figure 4. The superficial muscles of the head and neck. (Standring et al., 2008, modified)

5.3.1 MUSCLES OF THE UPPER FACE

Occipitofrontalis

Occipitofrontalis covers the dome of the skull from the highest nuchal lines to the eyebrows. It is innervated by the posterior auricular branch of the facial nerve and the frontal part is supplied by the temporal branches of the facial nerve.

The frontal parts raise the eyebrows and the skin over the root of the nose, acting from above, like in expressions of surprise or horror.

Corrugator supercilii

This muscle is located at the medial end of each eyebrow, lying deep to the frontal part of occipitofrontalis and orbicularis oculi, with which it is partially blended. The innervation is given by temporal branches of the facial nerve.

Corrugator supercilii cooperates with orbicularis oculi to draw the eyebrows medially and downwards to shield the eyes in bright sunlight. It is also involved in frowning.

Figure 5. Muscles of facial expression (1) Orbicularis Oris. (2) Levator labii superioris alaeque nasi. (3) Levator labii superiori. (4) Zygomaticus minor. (5) Levator anguli oris. (6) Zygomaticus major (7) Buccinator. (8) Depressor labii inferioris. (9) Depressor anguli oris. (10) Mentalis. (11) Orbicularis oculi. (Rizzolo and Madeira, 2005, modified)

Procerus

It arises from a fascial aponeurosis attached to the periosteum covering the lower part of the nasal bone, the perichondrium covering the upper part of the lateral nasal cartilage and the aponeurosis of the transverse part of nasalis. It is inserted into the glabellar skin over the lower part of the forehead between the eyebrows.

It is innervated by temporal and lower zygomatic branches from the facial nerve.

Procerus draws down the medial angle of the eyebrow and produces transverse wrinkles over the bridge of the nose. It is active in frowning and ‘concentration', and helps to reduce the glare of bright sunlight.

Orbicularis Oculi

Orbicularis oculi is a broad, flat, elliptical muscle which surrounds the circumference of the orbit and spreads into the adjacent regions of the eyelids, anterior temporal region, infraorbital cheek and superciliary region (Fig. 5 [11]).

The temporal and zygomatic branches of the facial nerve perform its innervation. This muscle is the sphincter muscle of the eyelids and plays an important role in facial expression and various ocular reflexes.

5.3.2 MUSCLES OF THE MIDFACE

Nasalis

Nasalis muscle consists of transverse and alar components. The transverse part (compressor naris) is attached to the maxilla above and lateral to the incisive fossa, and lateral to the alar part. The alar part (pars alaris or dilator naris posterior) is attached to the maxilla above the lateral incisor and canine, lateral to the bony attachment of depressor septi, and medial to the transverse part, with which it partly merges. The innervation of nasalis muscle is performed by the buccal branch of the facial nerve. It may also be supplied by the zygomatic branch of the facial nerve.

The transverse part compresses the nasal aperture at the junction of the vestibule and the nasal cavity. The alar parts draw the alae and posterior part of the columella downwards and laterally and so assist in widening the nares and in elongating the nose. They are active immediately before inspiration.

Levator Labii Superioris Alaeque Nasi

It is attached to the upper part of the frontal process of the maxilla, then descends inferolaterally, dividing into a medial slip attached to the greater alar cartilage and the skin over it and a lateral slip prolonged inferolaterally to blend with levator labii superioris and orbicularis oris (Fig. 5 [2]). The innervation is given by zygomatic and superior buccal branches of the facial nerve. The lateral slip raises and everts the upper lip and raises, depends and increases the curvature of the nasolabial furrow's superior part; the medial slip dilates the nostril and displaces laterally and modifies the curvature of the inferolaterally convex circumalar furrow.

Levator Labii Superioris

It descends from the inferior orbital margin, being attached to the maxilla and zygomatic bone above the infra-orbital foramen, and converges into the upper lip between the lateral slip of elevator labii superioris alaeque nasi and zygomaticus minor with, more deeply, levator anguli oris (Fig. 5 [3]). Levator labii superioris is innervated by the zygomatic and buccal branches of the facial nerve, and it elevates and events the upper lip.

Zygomaticus Minor

It is attached to the zygomatic bone behind the zygomaticomaxillary suture, descends medially into the upper lip; separated superiorly from levator labii superioris by a narrow triangular interval, but inferiorly blends with this muscle (Fig. 5 [4]). It is innervated by the zygomatic and buccal branches of the facial nerve, and elevates the upper lip exposing maxillary teeth and assists in deepening and elevating the nasolabial furrow. With the other main elevators, it curls the upper lip in smiling and in smugness, contempt or disdain.

Zygomaticus major

It extends from the zygomatic bone, in front of the zygomaticotemporal suture, to the modiolus near the buccal angle, blending here with levator anguli oris and orbicularis oris and also, more deeply, with order modiolar muscles (Fig. 5 [6]). The innervation is performed by the zygomatic and buccal branches of the facial nerve. Zygomaticus major retracts and elevates the modiolus and buccal angle, as in laughing. It is also a fixator of the modiolus.

Levator Anguli Oris

It is attached to the canine fossa below the infra-orbital foramen, whence it converges and mingles near the buccal angle (at the modiolus) with zygomaticus major, depressor anguli oris and other muscular bands including orbicularis oris. (Fig. 5 [5]). Zygomatic and buccal branches of the facial nerve perform its innervation. It raises the modiolus and buccal angle, incidentally displaying the teeth in smiling, and contributes to the depth and contour of the nasolabial furrow.

Buccinator

It is attached linearly to the external surfaces of the alveolar processes of maxilla and mandible, opposite the molar teeth; curving posteromedially around the sites of the third molar teeth, the upper part crosses the maxillary tuberosity. Inferiorly it attaches at the junction of the ramus and body of the mandible joint to the posterior end of mylohyoid line. Posteriorly it insets at the anterior border of pterygomandibular raphe, which is interposed between it and the superior pharyngeal constrictor (Fig. 5 [7]). Buccinator muscle is innervated by the buccal branch of the facial nerve. This muscle compresses the cheeks against the teeth, passing food between them in mastication, or expelling air when the cheeks are distended. Its labial extensions are mentioned below.

Orbicularis Oris

Actually this muscle consists of four substantially independent quadrants (upper, lower, left and right), each of which contains a larger pars peripheralis and a smaller pars marginalis (Fig. 6 [A, B, C, D]). This muscle is innervated by the buccal and mandibular branches of the facial nerve (Fig. 5 [1]).

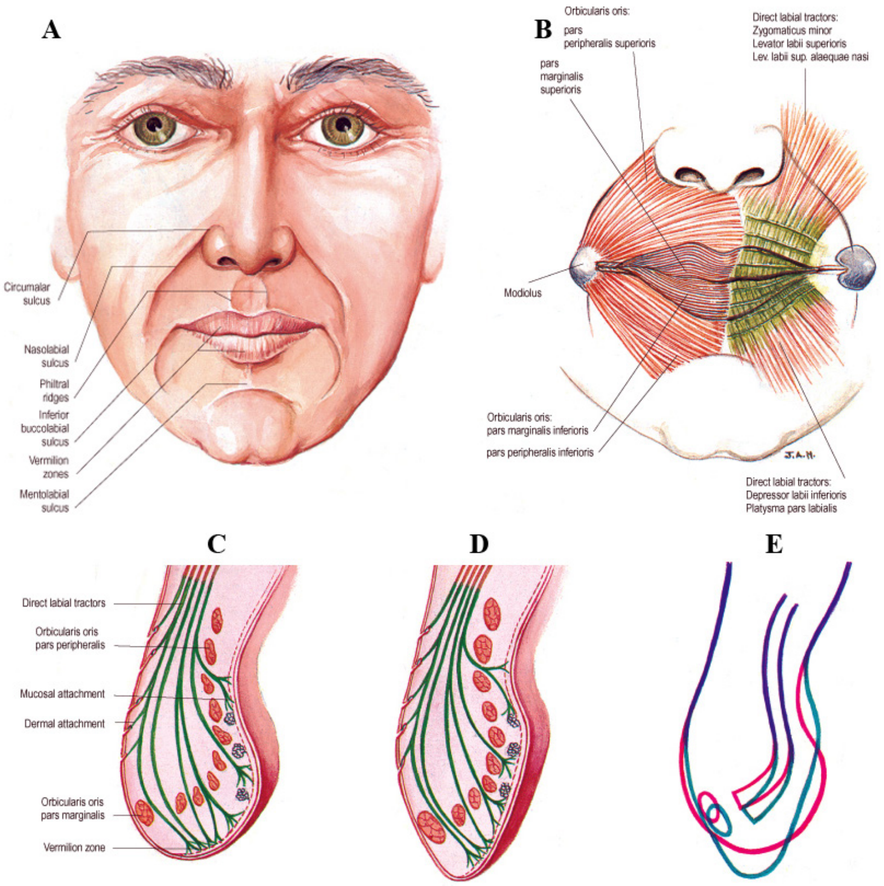


Figure. 6. (A) Principal sulci, creases and ridges of the face. (B) The disposition of the modiolus and orbicularis oris pars peripheralis and pars marginalis. (C) Parasagittal section of the upper lip in repose. (D) As C but slightly contracted forming a narrowed profile. (E) Superimposed outlines of C and D. (Standring et al., 2008, modified).

Risorius

Risorius is a highly variable muscle that ranges from one or more slender fascicles to a wide, thin superficial fan. The buccal branches of the facial nerve give itsinnervation. Muscle risorius pulls the corner of the mouth laterally in numerous facial activities, including grinning and laughing.

5.3.3 MUSCLES OF THE LOWER FACE AND NECK

Depressor Labii Inferioris

This quadrilateral muscle isattached to the mandibular oblique line, between symphysis menti and the mental foramen, ascending medially into the skin and mucosa of the lower lip, blending and intersecting with its contralateral and with orbicularis oris. It is continuous below and laterally with platysma (pars labialis); its superficial part contains some admixed fat but its overlying panniculus adiposus is very thin (Fig. 5 [9]). It is innervated by the mandibular branch of the facial nerve. It depresses the lower lip laterally in mastication, may assist its eversion and contributes to expressing irony, sorrow, melancholy, doubt etc.

Depressor Anguli Oris

It ascends from the mandibular mental tubercle and its continuation, the oblique line, inferolateral to depressor labii inferioris, and then converges into a narrow fasciculus blending with others muscles at the modiolus near the buccal angle. (Fig. 5 [8]). The buccal and mandibular branches of the facial nerve perform the innervation. It depresses the modiolus and buccal angle laterally in opening the mouth and in expressing sadness. During opening of the mouth the buccolabial sulci are stretched, flattened and become indistinct; the mentolabial sulcus becomes more horizontal and its central part deepened.

Mentalis

A conical fasciculus lying lateral to the inferior labial frenulum, it is attached in the mandibular incisive fossa and descends to the mental skin (Fig. 5 [10]). Mentalis is innervated by the mandibular branch of the facial nerve. It raises the mental tissues, mentolabial sulcus, (wrinkling the mental skin) and base of the lower lip, aiding its protrusion / eversion, as in drinking, speech and also expressing doubt or distain.

Platysma

Platysma is described as a muscle of the neck but it is considered here as a contributor to the orbicularis oris muscle complex; this muscle arises from the fascia over the upper chest and clavicle and extends over the anterolateral neck to meet in the midline at the lower chin margin. It has mandibular, labial and modiolar parts.

5.4 LABIAL STRUCTURE

The almost endless variety of neuromuscular controls of the lips and oral fissure during speech and non-verbal expressive communication are commonly integrated with fluctuating patterns in the masticatory, lingopharyngeal, laryngeal, circumnasal, circumorbital and palpebral, and so intraocular and extraocular muscle groups.

When the face is in repose, the lips are in gentle contact and the teeth maintaining a narrow interocclusal clearance, describing an approximately hexagonal area(borders: superior, inferior, paired superolateral and inferolateral).

The superior border is between the attached margin of the lower external nose and the upper lips and includes the bilateral curved circumalar sulci, the ridge forming the posterior rim of each nostril; it then continues beneath the junction of the anterior nasal spine of the maxillae and the mobile part of the nasal septum.

The superolateral boundaries, described above, incline downwards and laterally from the upper end of the circumalar sulcus to the modiolus and correspond to the so-called nasolabial sulci (or superior buccolabial sulci).

The inferolateral boundaries extend downwards and medially from the lateral angles of the hexagon over the modioli to the down-curved lateral ends of the centrally transverse mentolabial sulcus, the latter forming the inferior boundary. A transverse line between the external angle (i.e. between the equilibrium ‘resting’ positions of the modioli) separates the area, and crosses the level of the usually slightly undulant line of the contact between apposed free ‘red-lip’ surfaces at the closed oral fissure. A considerable variation (between individuals, sexes and races) in the dimensions and curvatures of the exposed red-lip surfaces is commonplace.

5.5 MOVEMENTS OF THE LIPS

The various groups of direct labial tractors may act together or individually, and their effects may involve a complete labial quadrant, or be restricted to a short segment. For example, partial contraction of the superior labial tractors can result in localized elevation of a segment of the upper lip, in a postural expression reminiscent of the "canine snarl".

Normally, however, the activity of the tractors is modified by the superimposed activity of orbicularis oris and the modiolar muscles. The resultant actions range from delicate adjustments of the tension and profile of the lip margins to large increases of the oral fissure with eversion of the lips.

Lip protrusion is passive in its initial stages. It may be suppressed by powerful contraction of the whole of orbicularis oris or enhanced by selective activation of parts of the direct labial tractors.

However, lip movements must accommodate separation of the teeth brought about by mandibular depression at the temporomandibular joints. Beyond a certain range of mouth opening, labial movements are almost completely dominated by mandibular movements. Thus over the last 2.5–3 cm interincisal distance of wide jaw separation, strong contraction of orbicularis oris cannot effect lip contact, and instead it causes full-thickness inflection of upper and lower lips, including the vermilion zone, towards the oral cavity, wrapping them around the incisal edges, canine cusps and premolar occlusal surfaces.

Contraction of marginalis is considered to alter the cross-sectional profile of the free margin of the vermilion zone such that both the gentle bulbous profile of the upper lip and the smooth posterosuperior convexity of the lower lip change to a narrow, symmetrical triangular profile. The transformed rims, whose length and tension can be delicately controlled, have been named labial cords. They are known to be involved in the production of some consonantal (labial) sounds. A labial cord may also function as a 'vibrating reed' in whistling or playing a wind instrument such as the trumpet.

5.6 ANATOMY OF SPEECH

5.6.1 OVERVIEW OF SPEECH PRODUCTION

All speech requires an input of energy. For all sounds in Western European languages, and most sounds in other languages, this energy takes the form of a pulmonary expiration. This continuous airflow is converted into a vibration within the larynx by a mechanism called phonation, in which the vocal folds vibrate periodically, interrupting the column of air as it leaves the lungs and converting it into a series of discrete puffs of air. Speech sounds that are produced by vocal fold vibration in this way are said to be voiced. Speech sounds that are produced without vocal fold vibration are termed unvoiced sounds.

Amplification and modification of the sound occur in the supralaryngeal vocal tract, narrow at the larynx and broadening out proximally as it passes through the pharynx, and oral and nasal cavities. This tube acts as a passive amplifier of the sound. The supralaryngeal vocal tract modifies the basic vibration of the larynx by altering its geometry, length and calibre: it provides a series of resonators that can dampen or amplify certain sound frequencies and can transiently interrupt the exhaled air flow and modify it to produce speech. This process is known as articulation. The range of sounds that the human vocal tract is capable of producing is very wide, although any one human language will employ a subset of these sounds to convey meaning.

5.6.2 ARTICULATION

The sound produced by the phonation is not a pure tone because several harmonics at multiples of the fundamental frequency are also generated. In the human vocal tract, the fundamental frequency and its harmonics are transmitted to the column of air which extends from the vocal cords to the exterior, mainly through the mouth. Part of the airstream can also be diverted through the nasal cavities when the soft palate is depressed to allow air into the nasopharynx. The supralaryngeal vocal tract acts as a selective resonator whose length, shape and volume can be varied by the actions of the muscles of the pharynx, soft palate, fauces, tongue, cheeks and lips; the relative positions of the upper and lower teeth, which are determined by the degree of opening and protrusion or retraction of the mandible; and alterations in the tension of the walls of the column, especially in the pharynx. Thus, the fundamental frequency (pitch) and harmonics produced by the passage of air through the glottis are modified by changes in the supralaryngeal vocal tract.

During articulation the egressive airstream is given a rapidly changing specific quality by the articulatory organs, the lips, oral cavity, tongue, teeth, palate, pharynx, and nasal cavity. In order to analyse the way in which the articulators are used in different speech sounds, words are broken down into units called phonemes, which are defined as the minimal sequential contrastive units used in any language.

The human vocal tract can produce many more phonemes than are employed in any one language. Not all languages have the same phonemes, and within the same language, the phonemes can vary in different parts of the same country and in other countries where that language is also spoken. Reproducing phonemes that are not used in native speech is difficult because such phonemes require unfamiliar positioning of the speech organs.

5.6.3 PRODUCTION OF VOWELS

All vowel sounds require phonation by vibration of the vocal cords. The sounds of the different vowels are determined by the shape and size of the mouth, and the positions of the tongue and lips are the most important variables. The tongue may be placed high or low (close and open vowels), or further forwards or back (front and back vowels) and the lips may be rounded or spread.

5.6.4 PRODUCTION OF CONSONANTS

The production of consonants always involves some degree of constriction of the vocal tract. There are many more consonants than vowels, and, in general, consonants cannot be combined to produce syllables.

Consonants may be classified of the basis of where the constriction occurs, termed the place of articulation; the degree or extent of constriction, termed the manner of articulation; the shape of the constriction, termed the stricture; and whether or not there is vibration of the vocal folds, when consonants are described as voiced or unvoiced respectively.

Consonants may also be classified as labial, dental, alveolar, velar, uvular, pharyngeal or glottic, depending upon whether the point of maximum constriction occurs at the level of the lips, teeth, bony ridge behind the teeth, palate, uvula or pharynx (Fig. 7). Different parts of the tongue can be used in combination with the above places of articulation. Stricture describes the shape of the constriction, e.g. a lateral consonant involves depression of the sides of the tongue, while a grooved consonant is produced by grooving the dorsum of the tongue. Consonants can be produced with the vocal folds vibrating, when they are termed voiced, or without vocal fold vibration, in which case they are termed unvoiced.

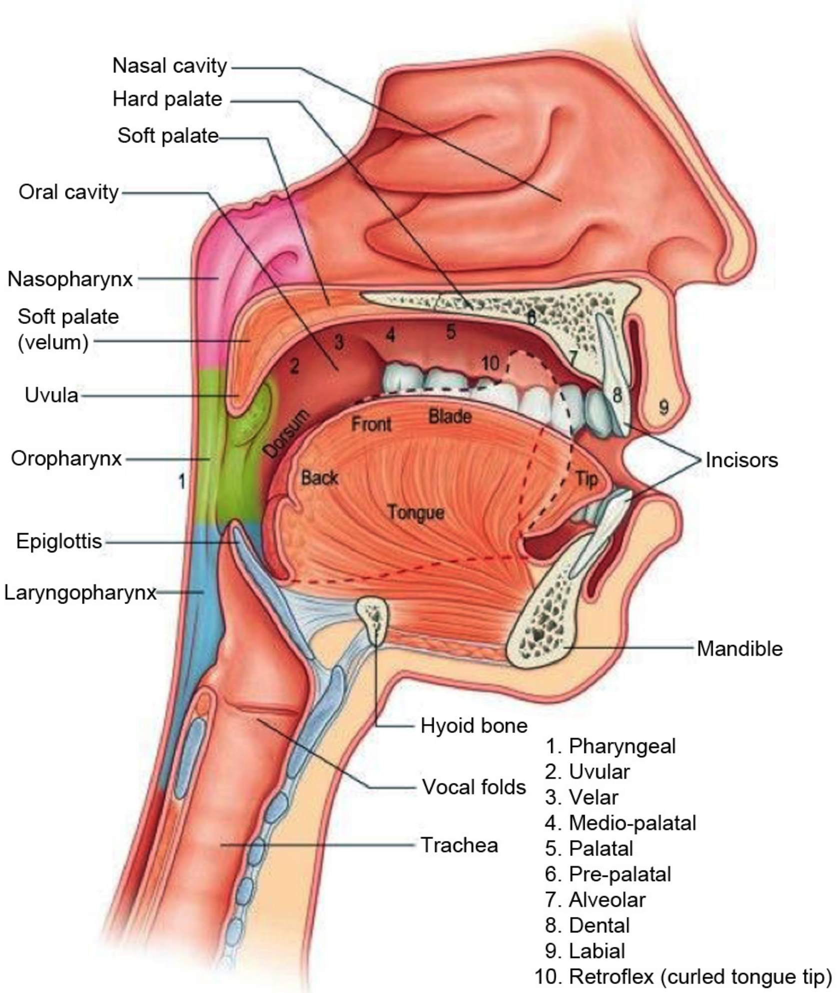


Figure 7. Sagittal view of the left side of the head, showing the supralaryngeal vocal tract, the articulators and places of articulation. The broken line indicates the tongue position during retroflexion (10). (Standring et al., 2008, modified).

6 MATERIAL AND METHODS

6.1 CLINICAL EXAMINATION

Subjects were examined while sitting on a dental chair in a room with appropriate lighting, by the same examiner, specialist in temporomandibular joint disorders (TMD) and orofacial pain. Data considered in the present study referred to tenderness to palpation in the masseter, temporal and supra-hyoid muscles and in the temporomandibular joint (TMJs). The subjects were asked to grade their pain using a printed numerical scale from zero (absence of pain) to 10 points (greatest pain possible).

The TMJ region was also palpated during the mandibular movements for the identification of joint noises, which were confirmed by auscultation. The Research Diagnostic Criteria for TMD (RDC/TMD - axis I, Dworkin and LeResche, 1992) was used for classification.

6.1.1 SELF-JUDGEMENT OF SEVERITY

The ProTMDmulti questionnaire was used to determine the perception (presence and severity) of TMD signs and symptoms by the subjects. The questionnaire is divided into two parts; the first part asks about the presence of TMD signs and symptoms with a series of 12 questions requiring a positive or negative reply. In the second part, the subjects were asked to indicate the severity of nine signs and symptoms present (or no) according to the situation, i.e. when waking up, during mastication, when speaking and at rest. Severity was indicated on a printed 11-point numerical scale where zero corresponded to the complete absence of the symptom, and 10 corresponded to the highest possible severity. The severity score was the sum of the scores attributed to each sign and symptom in the four questioned situations. The severity score varies between zero (absence) and 40 (the highest possible severity) (Felício et al., 2009a).

6.1.2 OROFACIAL MYOFUNCTIONAL EVALUATION

The components of the stomatognathic system were evaluated in terms of mobility according to the Orofacial Myofunctional Evaluation with Scores Protocol (OMES – Part I) (Felício and Ferreira, 2008) when the healthy subject was asked to perform the following movements:

- Lips: protrusion, retrusion, lateral to the right and left;
- Tongue: protrusion, retrusion, lateral to the right and left, raising, and lowering and ability to keep the tongue in stable protrusion for 5 seconds;
- Mandible: protrusion, lowering, raising, lateral to the right and left.

In the analysis, separate movements of each component were considered normal if precise and without tremors. Dysfunction was considered present when lack of precision in the movement, tremor, associated movements of other components (e.g., lips accompanying the movements of the tongue) or inability to perform the movement was observed.

According to the OMES Protocol, the examiner attributed scores on a 3 point scale: 3 = normal; 2 = insufficient ability; 1 = absence of ability or being unable to perform the task.

To complement the analysis for jaw movements, measurements (in mm), symmetry/asymmetry during mouth opening and closing, right and left laterality and protrusion will also be considered. Scores were attributed according to the protocol.

6.2 SUBJECTS

Twenty healthy young adults (10 men and 10 women) aged 20 to 41 years, natural speakers of Italian language, participated in the study. They were recruited from the students and staff attending the Department of Biomedical Sciences for Health, University of Milan. All subjects had a clinically normal facial function, no previous facial trauma, paralysis or surgery, no known neurological diseases, and no and current orthodontic treatment.

To be recruited, the healthy subjects had no to present TMD according to the Research Diagnostic Criteria for TMD (RDC/TMD, Dworkin and LeResche, 1992), to the ProTMDmulti protocol (Felício et al, 2009a), and an orofacial myofunctional evaluation (Felício and Ferreira, 2008), as detailed in Chapter 5.1.

6.3 INSTRUMENTATIONS

6.3.1 OPTOELECTRONIC MOTION ANALYZER

Lip movements in verbal and non-verbal activities were recorded using an optoelectronic three-dimensional motion analyzer, the SMART-E system (BTS S.p.a, Garbagnate Milanese, Italy).

High-precision infrared sensitive CCD video cameras (Fig. 8) are coupled with the video processor with up to 120 Hz sampling ratio. The 3D positions of lightweight, passive and retro-reflective markers are instantly recorded with a spatial accuracy of up to 0.1 mm.

Figure 8. Detail of a camera.

In brief, stroboscopic infrared light (wavelength, 880 nm) is emitted by an array of LED (light emitting diodes) mounted around the lens of each camera, and the CCD sensor detects the reflection from the markers placed on the body.

The process of recognizing passive markers in the 2D video frames is performed via enhanced blob analysis. The 3D coordinates of each marker are finally computed based upon the 2D data of at least two cameras. This process, called spatial triangulation, needs the system to be previously calibrated. Calibration allows the system to estimate the capture volume, the relative position and orientation of the cameras (external parameters), their geometric and optical characteristics (internal parameters).

The BTS SMART system requires two calibration phases. The “static calibration” sets the position and the orientation of the global reference system: all cameras simultaneously record a still, special reference device (Fig. 9), whose marker reciprocal distances are known to the system. The "dynamic" calibration exploits the epipolar constraint between a 3D point and its 2D projections on the sensor of two cameras: all cameras simultaneously record a rigid bar (Y axis) in motion throughout the working volume.

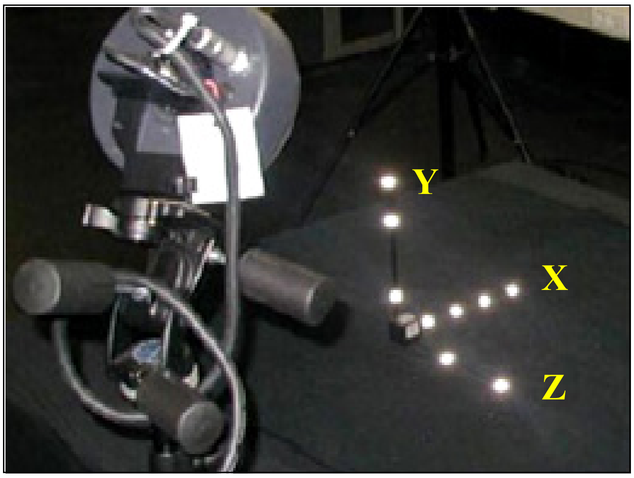
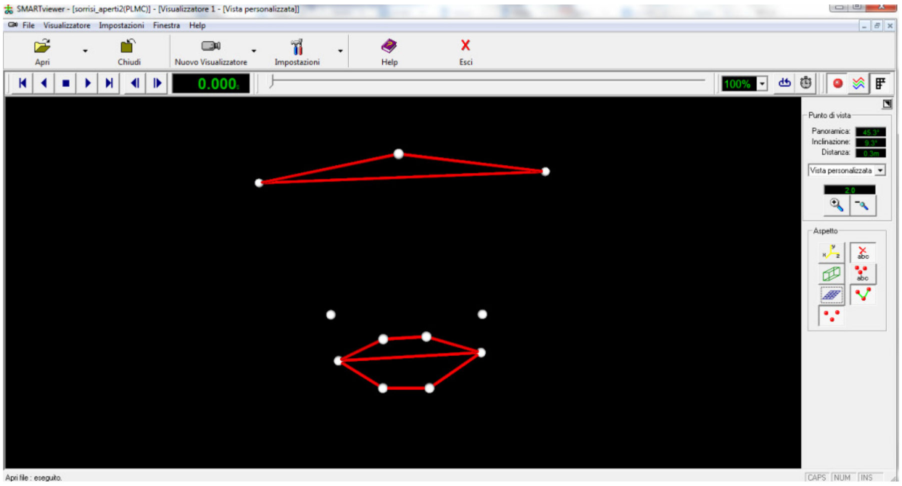


Figure 9. Global reference system.

At the end of the metric calibration and correction of optical and electronic distortions, the system provides the current accuracy level, which will characterize the following acquisition sessions.

Once a movement has been recorded, special software provides the spatial configuration of the marker set (Fig. 10).


SMARTviewer - [sorrisi_aperti2(PLMC)] - [Visualizzatore 1 - [Vista personalizzata]]

Panoramica: 45.3°
Inclinazione: 9.3°
Distanza: 0.3m
Vista personalizzata
2.0

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Figure 10. 3D graphic representation of the marker set at issue.

The operator has to label the markers of interest in one frame, opening the corresponding model previously created; afterward, the system should be able to recognize all moving markers, tracking their pathways.

To record lip movements (Ferrario and Sforza et al., 2007; Sforza et al., 2010b,c; 2012), nine cameras were deployed around a stool (Fig. 11), and calibrated to create a 60 (width) cm x 60 (height) cm x 60 (depth) cm working volume; metric calibration and correction of optical and electronic distortions are performed before each acquisition session using a 20-cm wand, with a resulting mean dynamic accuracy of 0.121mm (SD 0.086), corresponding to 0.0158% (Sforza et al., 2010c). A 60 Hz capture rate was used for all acquisitions.

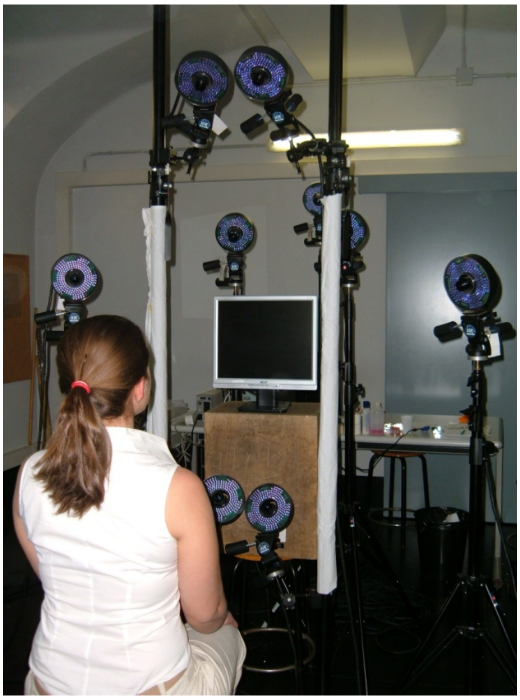

Figure 11. Setting of the cameras with respect to the subject sat on a stool.

6.3.2 ELECTROMYOGRAPHIC SYSTEM

The BTS FREEEMG system (BTS S.p.a, Garbagnate Milanese, Italy) is a wireless electromyographic device with active probes weighting just 8 grams for signal acquisition and wireless transmission (Fig. 12). The probes amplify the differential EMG signals captured by disposable pre-gelled silver/silver chloride bipolar surface electrodes, digitize them and communicate with a portable receiving unit. The complete absence of cables allows for quick and comfortable preparation of the subject, without affecting in any way the motor pattern. This system is easily connectable with the motion analyzer, permitting the real time recording of synchronized kinematic and electromyographic data.

Figure 12. Detail of an EMG probe clipped on a pair of electrodes.
(http://www.btsbioengineering.com/BTSBioengineering/Surfaceemg/BTSFREEEMG300/BTS_FREEEMG300.html, last accessed 11 December 2012).

In surface EMG, a bipolar probe measures the voltage difference between two electrodes, which is the sum of the electrical contributions of the active motor units; thus it reflects both the muscle membrane properties and the central control strategies. With small inter-electrode distance with respect to the muscle size, the activity conducted from adjacent muscles is similar on the two leads, and therefore is partly rejected (Castroflorio et al., 2008).

6.3.3 RECORDING PROTOCOL

For each subject, the recordings took approximately 40 minutes (considering also the time needed for subject's preparation). The protocol did not involve dangerous or painful procedures, and it was preventively approved by the ethics committee of the Department of Biomedical Sciences for Health, University of Milan.

After the methods and aims of the investigation had been completely described, written informed consent was obtained from each participant.

Kinematic assessment of speech and lip movements

Subjects sat on a stool inside the working volume and were asked to perform a series of standardized lip movements and speech pronunciation. During the execution of the movements, for each camera special software detected the two-dimensional coordinates of facial landmarks identified by a set of 2-mm round reflective markers. Landmarks were chosen from classic anthropometry (Farkas, et al., 1994): *n*, nasion; *ft*, right and left frontotemporale; *ng*, right and left naso-genian; *cph*, right and left crista philtri; *ch*, right and left cheilion; *li*, right and left lower lip midpoints (Figs. 13, 14). The positions of the markers were carefully controlled to avoid any interference with lip and speech movements (Trotman et al., 2003; Hontanilla and Aubá, 2008; Sforza et al., 2010b,c; 2012). Subsequently, all the coordinates were converted to metric data, and a set of three-dimensional coordinates for each landmark in each frame that constituted each movement was obtained.

Each animation was explained and shown to the subjects, which practiced before data acquisition. For each expression, each subject performed ten standardized maximum facial expression from rest (Wachtman et al., 2001; Hontanilla and Aubá, 2008; Mehta et al., 2008; Sforza et al., 2010b,c), without modifications of the markers positions.

The healthy subjects performed four standardized non-verbal movements: open mouth smile, closed mouth smile, spontaneous smile and lip purse; and verbal movements: natural and random (e.g. o, a, i, u, e) sequence of the five vowel, a sequence of natural numbers (e.g. 1 to 10) and 29 Italian words (Table 1).

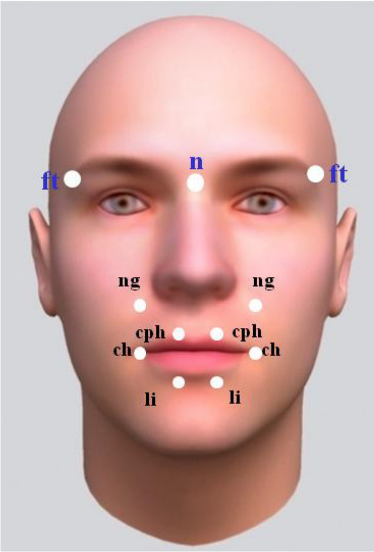

Figure 13. Position of the reflective markers. *(n)* nasion, *(ft)* right and left frontotemporale, *(ng)* right and left naso-genian, *(cph)* right and left crista philtri, *(ch)* right and left cheilion, *(li)* right and left lower lip midpoints.

Electromyographic assessment of speech and lip movements

During the EMG recording, the environment must be calm, quiet and with little luminosity. The subject was seated on a chair, with erect posture and with both feet on the ground and both arms leaning on the legs. The head was positioned in an erect way, being the Frankfort plane considered as positioning parameter.

To reduce skin impedance, facial epidermis was carefully cleaned with alcohol prior to the 10 mm-electrodes placement (Kendall Arbo; Tyco Healthcare, Neustadt, Germany). The greater zygomatic major muscles and depressor labii inferioris from right and left sizes were examined with the positioning of four probes parallel to the muscular fibers of the relevant muscles (Fig. 14). The main criterion for determining the recording sites was to maximize the distance to the adjacent muscles in order to reduce crosstalk.

Figure 14. Position of the reflective markers and the electrodes of the BTS FREEEMG system (BTS S.p.a, Garbagnate Milanese, Italy).

All the necessary explanations were given previously, as well as the lip movements were practiced with the examiner prior to test performance.

The subjects were asked to perform 10 consecutive voluntary open and closed-mouth smiles, and a spontaneous smile, while watching a fun film. The resting time between the open and closed smiles was about 3 seconds (Fig. 15).

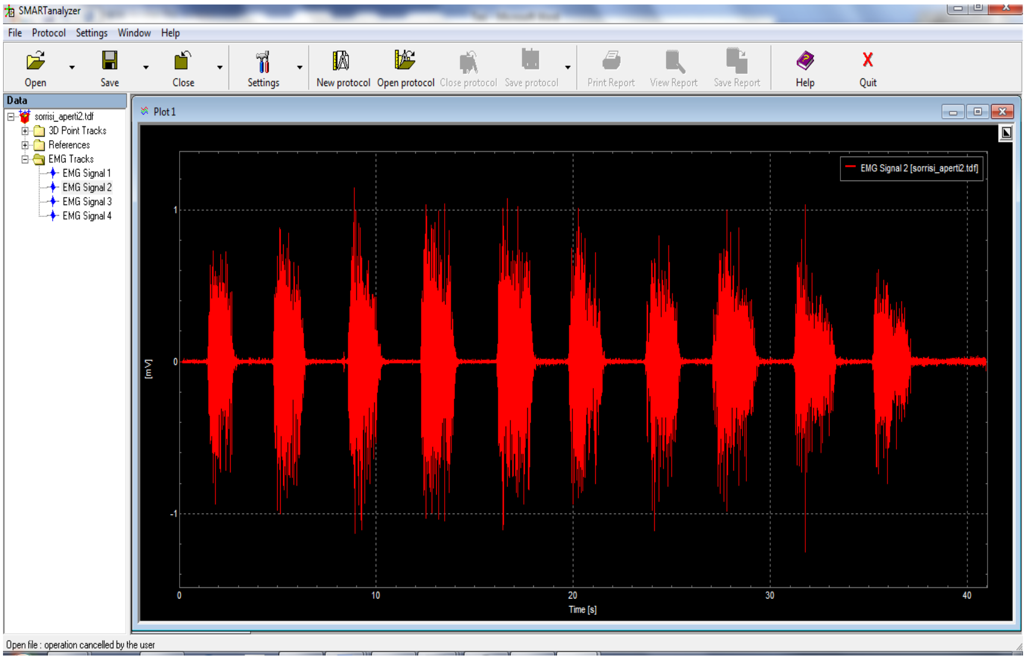

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Figure 15. Raw EMG signal of the right zygomatic major muscle during open-mouth smile.

For verbal movements they were asked to pronounce a sequence of natural numbers (eg. 1 to 10), and 29 Italian words (Table 1).

Table 1. Italian words pronunciation.

Italian words pronunciations		
1. Panico	11. Velocità	21. Arrivederci
2. Logopedista	12. Nocivo	22. Caricatura
3. Terapia	13. Distanza	23. Scendiletto
4. Bevanda	14. Affascinante	24. Circolare
5. Riabilitazione	15. Significato	25. Imagine
6. Gamba	16. Isola	26. Criminologia
7. Metamorfosi	17. Rissa	27. Gnocchi
8. Gemma	18. Presidente	28. Ogni
9. Famiglia	19. Zinco	29.Foglia
10. Sceriffo	20. Zero	

6.3.4 MEASUREMENT PROTOCOL

Both raw EMG signals and marker coordinates constituted the input data for the protocol calculations, which were implemented on Microsoft Excel and Smart Analyzer. Descriptive and inferential statistics were evaluated by means of SPSS Statistics.

Kinematic of speech and lip movements

For each subject, head and neck motion was subtracted from the raw facial movements using the three cranial (reference) markers, so only movements occurring in the face (activity of mimetic muscles) were further considered. Subsequently, for each of the 8 facial markers, the three-dimensional movements during both verbal and non-verbal activities were computed, and the modulus (intensity) of the three-dimensional vector of maximum displacement from rest was calculated (Ferrario and Sforza, 2007; Sforza et al., 2010b,c; 2012). For the natural sequence of numbers and words production, instantaneous displacements was also calculated.

For each animation, the landmark (single or paired) with the largest displacement from rest was identified. For smile movements, the latero-lateral (right-left direction) component of the maximum displacement of the analysed landmarks was computed.

The frontal area of movement (XY planes; unit, mm^2) for smiles, lip purse and vowels is estimated with the perimeter defined by the 6 labial markers (cph, ch and li). The Δ area (unit, mm^2) was calculated between the areas of maximum expression and rest position.

To assess differential movements between the two hemi-faces, percentage indices of asymmetry were computed as:

(right side displacement - left side displacement) /
(right side displacement + left side displacement) × 100.

The indices were computed for the total movement, as well as for the single landmarks. The indices range between -100% (complete left-side prevalence during the movement) and +100% (complete right-side prevalence) (Linstrom et al., 2002; Sforza et al., 2010c; 2012).

For the kinematic analysis of the markers of number sequences and words pronunciation, data were low-pass filtered using a Butterworth filter with cutoff frequency at 8 Hz.

The percentage overlapping coefficient (POC, unit: %) for words and numbers was calculated as:

$$\mathrm{POC} = \left[1 - \Sigma \left|\text{right side displacement}_i - \text{left side displacement}_i\right| / \Sigma \left|\text{right side displacement}_i + \text{left side displacement}_i\right|\right] \times 100$$

($_i$ - *instantaneous*)

The index ranges between 0% (no symmetry) and 100% (perfect symmetry).

The total percentage indices of asymmetry for sequence of numbers and words pronunciation (ASYM, unit %) was computed as:

$$\mathrm{ASYM} = \text{mean}\,(\text{right side displacement}_i - \text{left side displacement}_i) / \text{mean}\,(\text{right side displacement}_i + \text{left side displacement}_i) \times 100$$

($_i$ - *instantaneous*)

The ASYM index ranges between -100% (left-side prevalence of pronunciation) and +100% (right-side prevalence of pronunciation).

Electromyographic signals of speech and lip movements

For each muscle the EMG signal was filtered with a Butterworth "high pass" filter at the cutoff frequency of 50 Hz. Then we calculated the root mean square (RMS) signal with a time window of 25 ms, which was “low pass” filtered at 5 Hz.

For each movement, a custom made algorithm allowed the semi-automatic detection of the beginning of the muscle activity, and the time of latency between the activities of the four muscles was calculated.

The time difference in the onsets of electrical activity between the right and the left side of the face is referred to as asymmetry. Asymmetry is related to either the zygomatic muscles or the depressor labii inferioris (Fig. 17). It is expressed in seconds (s). The time difference in the onsets of electrical activity between zygomatic and depressor labii inferioris muscles is referred to as asynchrony. Asynchrony is related to each side of the face separately, either right or left (Fig. 18). Like asymmetry, it is expressed in seconds (s).

For open-mouth, closed-mouth and spontaneous smiles, the time of activation, the asymmetry between the right and left zygomatic major or depressor labii inferioris muscles, and the asynchrony between the zygomatic major and depressor labii inferioris were analyzed.

For speech pronunciation (natural sequence of numbers, and words), we calculated only by the asymmetry between the right and left depressor labii inferioris muscles.

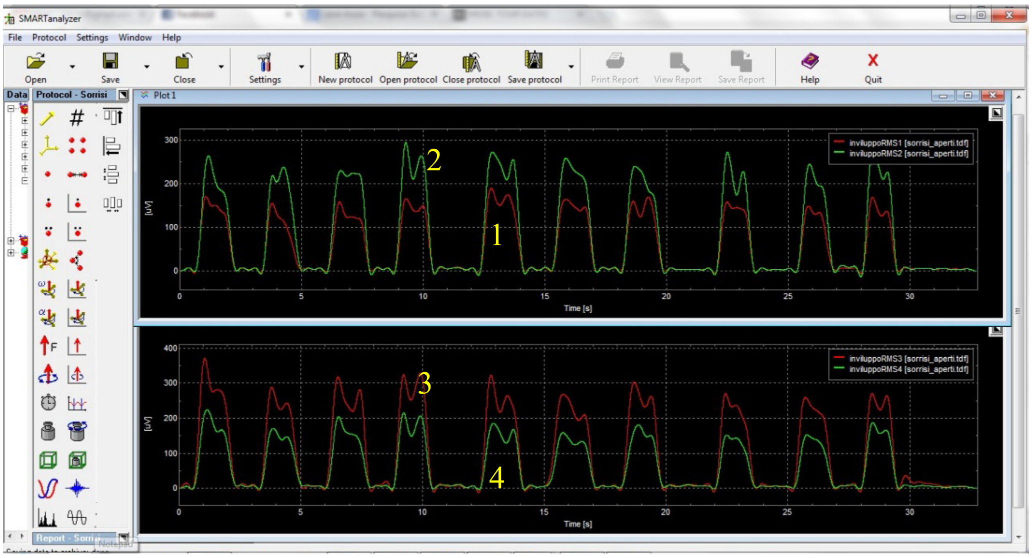

Figure 16. Asymmetry onset time of signal of the (1) right zygomatic major muscle, (2) left zygomatic major muscle, (3) right depressori labii inferioris muscle, and (4) left depressori labii inferioris muscle during open-mouth smile.

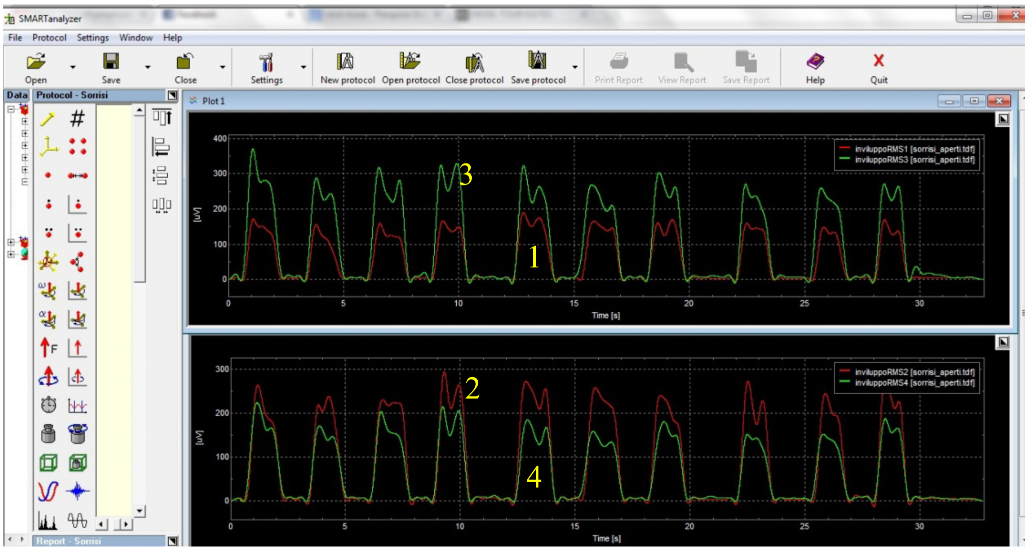

Figure 17. Asynchrony onset time of signal of the (1) right zygomatic major muscle, (2) left zygomatic major muscle, (3) right depressori labii inferioris muscle, and (4) left depressori labii inferioris muscle during open-mouth smile.

6.3.5 METHOD ERROR

For the optoelectronic three-dimensional motion analyzer, within- and between-session repeatability was previously assessed in healthy subjects. Within session, the technical error of the measurement for single landmarks (random error) was, on average, 0.5 and 3.38 mm, showing good reproducibility. Between sessions, all facial movements had standard deviations lower than 1mm (Sforza et al., 2010b,c).

For EMG analysis, the repeatability was assessed with hierarchical ANOVA, showing good reproducibility in the recorded smiles activities.

7 KINEMATICS ANALYSIS OF LIP MOTION IN HEALTHY SUBJECTS

7.1 METHODS

7.1.1 DATA COLLECTION

The kinematic indices calculated for verbal and non-verbal activities are summarized in table 2.

Table 2. Kinematics indices for verbal and non-verbal activities: *(cph)* crista philtri, *(ch)* cheilion, *(li)* lower lip midpoints.

	KINEMATIC INDICES					
	Asymmetry (%) (ch, cph, li, total)	Landmarks and Total Mobility (mm)	Frontal area (mm^2)	Δ area (mm^2)	POC (%)	ASYM (%)
Open-mouth smile	✓	✓	✓	✓		
Closed-mouth smile	✓	✓	✓	✓		
Spontaneous smile	✓	✓	✓	✓		
Lip purse	✓	✓	✓	✓		
Natural sequence vowels	✓	✓	✓	✓		
Random sequence vowels	✓	✓	✓	✓		
Natural sequence numbers					✓	✓
Italian words					✓	✓

7.1.2 STATISTICAL ANALYSIS

For all subjects, ten series of lip movements (open-mouth smile, closed-mouth smile, spontaneous smile, lip purse), natural and random sequences of vowels production were averaged, and the mean values of maximum marker displacement for each movement used for subsequent analysis. For the natural sequence of numbers and words production, the instantaneous displacements of landmarks was calculated.

Descriptive statistics were obtained for each marker, the total lip movements, the asymmetry indices, and frontal area, separately for each sex.

The normal distribution of data was checked with the Kolmogorov-Smirnov test.

The largest displacement from rest, total mobility and frontal area were compared between sexes by Student's t-Test for unpaired samples in all smiles and lip purse movements. To assess if the asymmetry indices significantly deviated from the expected value of 0, Student's t-tests for paired samples were made.

For natural and random sequences of vowels, the largest displacement from rest was compared between sexes by Student's t-Test for unpaired samples, and the total mobility was compared by three-way factorial analysis of variance (factors: sexes, side, and vowels pronunciation). For words and sequences of numbers, the POC and asymmetry indices were compared by two-way factorial analysis of variance (factors: sex and pronunciation).

The significance level was set at 5% for all statistical analyses ($p < 0.05$).

7.2 RESULTS

All data within each subgroup (men, women) were normally distributed (Kolmogorov–Smirnov tests, $p > 0.05$), and no significant difference was found between male and female mean ages (Student's unpaired t-test; all p-values > 0.05).

On average, during the execution of the non-verbal activities the cheilion landmarks had the largest displacements in both sex groups. For open-mouth smile and lip purse activities, the largest displacement from rest was similar between sex groups without statistically significant differences (Student's unpaired t-test; all p-values > 0.05). In contrast, men had significant larger movements than women during the execution of the closed-mouth smile (right crista philtri and left naso-genian landmarks, both $p = 0.04$), and the spontaneous smile (right and left lower lip, and left naso-genian landmarks, respectively, $p = 0.01$, $p = 0.03$ and $p = 0.02$) (Table 3).

Within open mouth smile, closed-mouth smile and lip purse movements, the total facial mobility was similar in both sexes, without statistically significant differences (Student's unpaired t-test; all p-values > 0.05). In contrast, in spontaneous smile sex-related differences were found on the left side ($p = 0.041$; Student's unpaired t-test): the male group performed this movement with larger landmark displacements than the female group (Fig. 18).

Table 3. Maximum displacement of single landmarks (mm) during the execution of non verbal activities: mean ± SD values in 10 women and in 10 men. (R –right; L – left; ng – naso-genian; *ch* – cheilion; *cph* – crista philtri; *li* - lower lip midpoints).

Landmarks (mm)	**Open mouth smile**				**Closed-mouth smile**				**Spontaneous smile**				**Lip purse**			
	Women		*Men*		*Women*		*Men*		*Women*		*Men*		*Women*		*Men*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ng R	7.2	2.3	8.1	2.2	5.2	2.3	7.4	2.6	7.4	2.1	9.8	3.7	5.6	1.6	5.9	2.7
ch R	16.5	4.5	14.3	3.8	11.3	3.6	13.2	4.1	14.8	5.0	17.2	5.8	11.2	2.4	12.6	2.3
cph R	8.3	3.4	8.5	2.6	4.3*	2.0	6.7*	2.8	8.3	2.8	10.9	3.6	8.2	1.9	9.2	1.8
li R	8.0	3.2	9.5	3.3	5.6	2.6	6.3	2.6	7.1*	2.8	11.5*	4.9	8.6	1.9	10.6	2.9
ng L	7.3	1.8	8.2	3.1	4.9*	1.8	7.3*	3.1	7.0*	2.3	10.8*	3.9	5.2	1.3	6.0	2.0
ch L	16.8	3.2	15.7	5.1	12.0	3.3	13.7	5.4	15.1	4.3	17.9	4.9	11.3	2.6	13.0	3.0
cph L	8.7	2.7	8.6	3.6	4.9	1.9	7.0	3.6	8.4	3.0	10.8	2.8	7.9	2.3	9.4	2.1
li L	8.0	2.9	9.7	3.5	5.6	2.5	6.7	2.7	6.9*	2.8	11.4*	4.2	8.5	2.1	10.1	2.6

* Significant differences, Student’s unpaired t-test (men vs women).

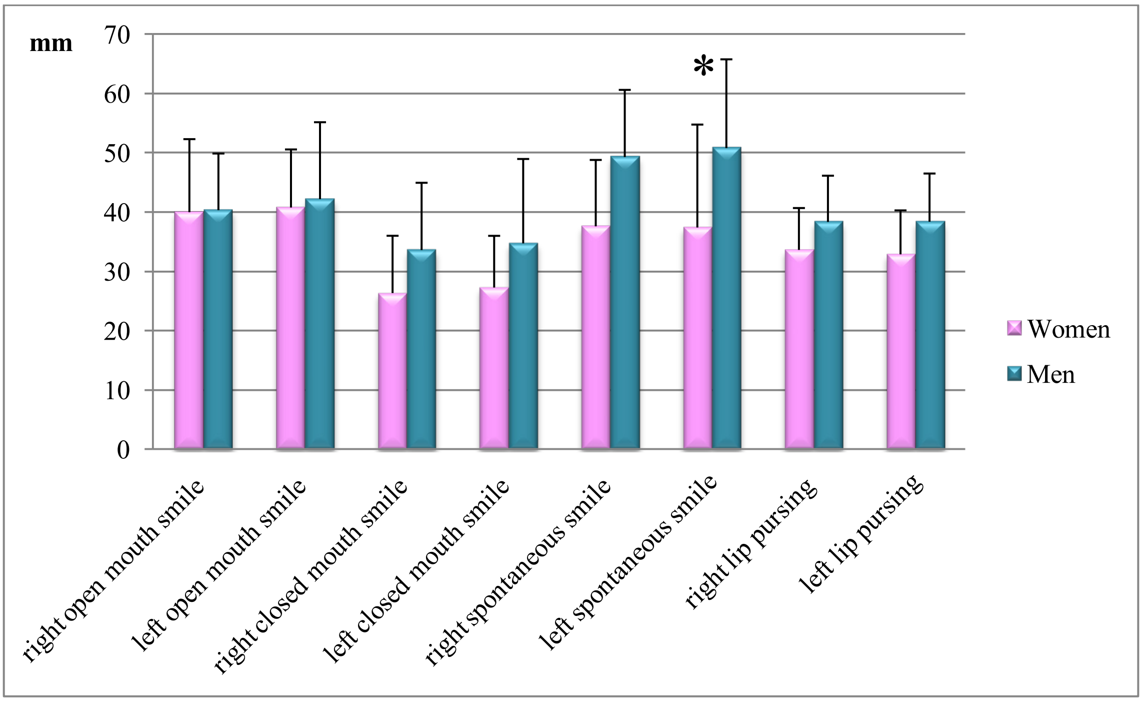

Figure 18. Total mobility in the four standardized lip movements (mean + 1SD).
* Significant differences on the left side in spontaneous smile, Student's unpaired t-test (men vs women).

The asymmetry of the upper lip landmarks was significantly different from 0 during closed-mouth smile in women (p = 0.033, Student's t-Test for paired samples), in the other movements (open-mouth smile, spontaneous smile and lip purse) no significant differences were found in asymmetry indices in all landmarks (cph, ch and li) and in total asymmetry (Fig. 19).

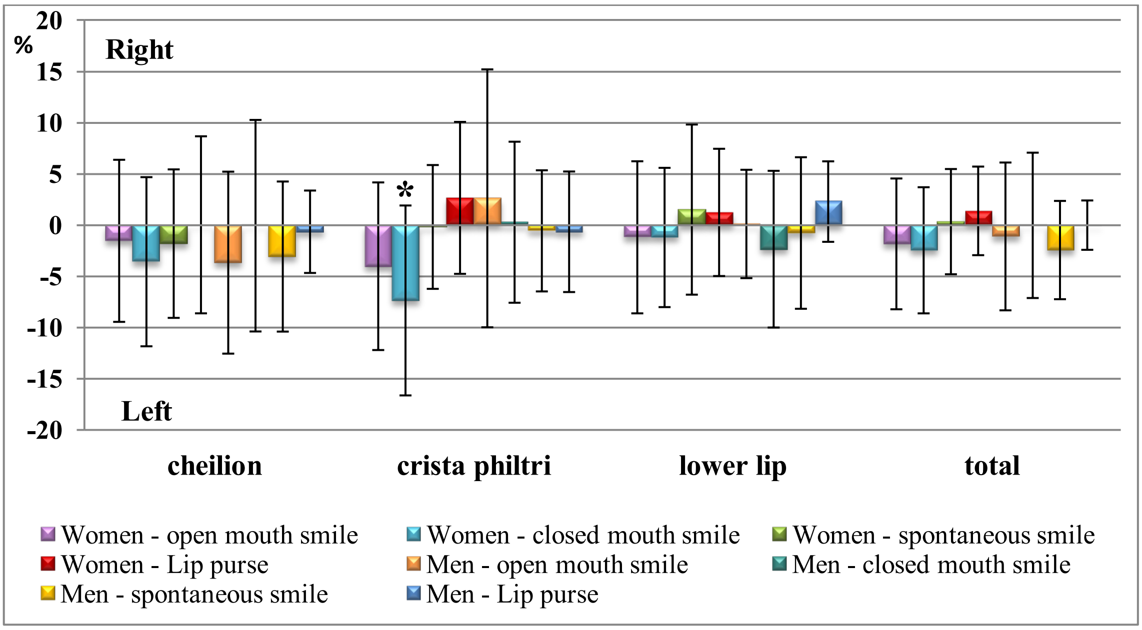

Figure 19. Mouth asymmetry indices for the four symmetric lip movements (mean ± 1SD).
* Significant difference in women during closed-mouth smile, Student's paired t-test.

The lateral displacement of labial commissure landmark (ch) was significantly different between sexes in open-mouth smile on the right side (p = 0.043; Student's unpaired t-test), while in the others smiles all labial landmarks (cph, li, and total) had no statistically significant differences between sexes (Fig. 20).

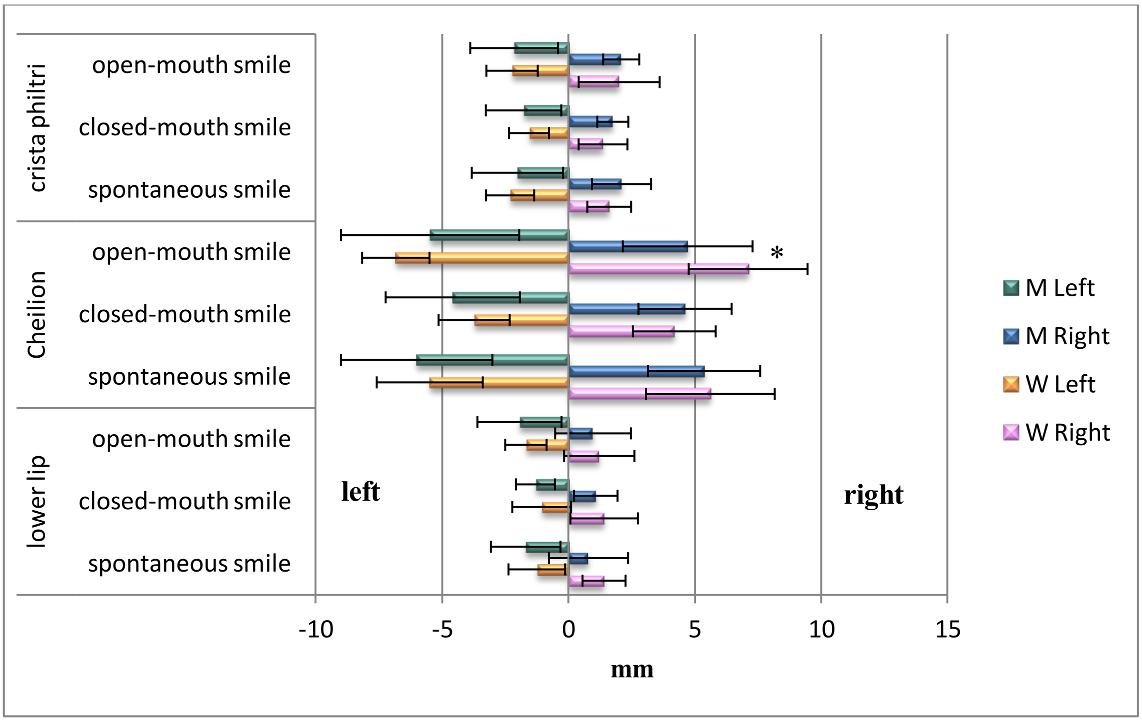

Figure 20. Smile activities: lateral displacement (right and left direction, mm) of labial landmarks (mean ± 1 SD). Positive displacements: right side prevalence; negative displacements: left side prevalence (M: men; W: women).

* Significant differences on the right side in open-mouth smile, Student's unpaired t-test (men vs women).

Total lip frontal area was larger in men than in women with significant differences in closed-mouth smile and spontaneous smile and lip purse activities (Table 4). In both sequences of vowels between sexes differences in frontal lip areas were found (Tables 6, 8) (Student's unpaired t-test).

In Δ frontal area, between sexes differences were found only for the random sequence pronunciation of vowel (u) (Student's unpaired t-test) (Tables 5, 7, 9).

Table 4. Frontal area (mm^2) in smiles and lip purse movements.

	Frontal area (mm^2)				
	women		*men*		t-test p-value
	mean	SD	mean	SD	
Open mouth smile	981.3	152.4	1059.9	166.5	NS
Closed mouth smile	540.2	70.0	734.7	144.2	p = 0.002
Spontaneous smile	874.7	228.5	1105.7	193.3	p = 0.0031
Lip purse	655.9	69.0	806.1	102.5	p = 0.001

NS, not significant, $p > 0.05$.

Table 5. Frontal Δ area (mm^2) in smiles and lip purse movements.

	Δ frontal area (mm^2)				
	women		*men*		t-test p-value
	mean	SD	mean	SD	
Open mouth smile	434.0	155.1	404.3	161.4	NS
Closed mouth smile	-12.8	77.9	76.7	149.4	NS
Spontaneous smile	311.5	235.3	442.4	159.9	NS
Lip purse	87.6	57.2	148.8	85.0	NS

NS, not significant, $p > 0.05$.

Table 6. Frontal area (mm^2) in vowels in natural sequence.

	Frontal area (mm^2)				
vowels	*women*		*men*		t-test p-value
	Mean	SD	mean	SD	
a	1089.9	233.6	1208.8	130.6	NS
e	918.6	146.7	1030.9	142.6	NS
i	837.1	76.2	991.0	146.9	p = 0.011
o	845.9	97.1	1011.9	107.4	p = 0.002
u	653.2	56.6	820.0	115.9	p = 0.001

NS, not significant, $p > 0.05$.

Table 7. Frontal Δ area (mm^2) in vowels in natural sequence.

	Δ frontal area (mm^2)				
vowels	*women*		*men*		t-test p-value
	Mean	SD	mean	SD	
a	530.9	252.4	554.4	110.5	NS
e	359.6	166.1	376.5	98.7	NS
i	278.1	74.2	336.6	104.0	NS
o	286.9	96.4	357.5	82.0	NS
u	94.2	52.4	165.7	92.4	NS

NS, not significant, $p > 0.05$.

Table 8. Frontal area (mm^2) in vowels in random sequence.

	Frontal area (mm^2)				
vowels	*women*		*men*		t-test p-value
	Mean	SD	mean	SD	
a	995.8	109.3	1166.0	62.7	0.001
e	878.5	122.9	1075.7	123.7	0.002
i	860.4	118.1	931.6	1041.2	0.010
o	853.1	122.7	1084.7	1031.2	0.001
u	666.0	40.1	829.0	846.9	0.002

Table 9. Frontal Δ area (mm^2) in vowels in random sequence.

	Δ frontal area (mm^2)				
vowels	*women*		*men*		t-test p-value
	Mean	SD	mean	SD	
a	434.2	119.9	501.4	82.4	NS
e	316.9	142.0	411.2	80.3	NS
i	298.8	125.4	376.6	131.7	NS
o	291.5	138.1	366.6	77.1	NS
u	104.4	65.7	182.4	82.3	0.032

NS, not significant, $p > 0.05$.

On average, during the execution of the natural and random sequences of vowels, the lower lip midpoint landmarks had the largest displacements in both sex groups. Significant sex-related differences of vowels pronunciation were found for the right cheilion landmarks for natural sequence of vowels (a) (p = 0.043), (e) (p = 0.040), and for left crista philtri landmark for random sequence of vowel (o) (p = 0.013) (Tables 10, 11).

For both sequences of vowel pronunciation (natural and random), the total mobility in vowels (o, i, u) was similar, without significant differences (three-way factorial analysis of variance; factors sex, vowels and sides, all p-values > 0.05). In contrast, significant side-related differences of vowels pronunciation were found for the vowels (a) (p = 0.010), and (e) (p = 0.007) (Fig. 21).

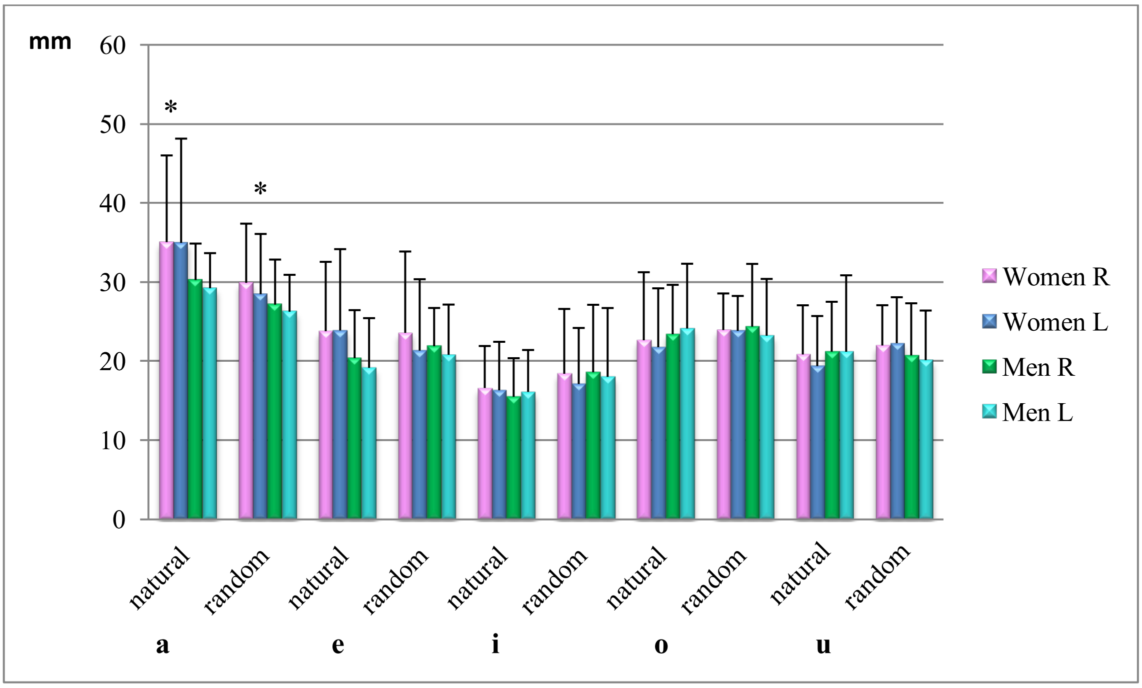

Figure 21. Total mobility in the natural and random sequences of vowels production (mean + 1SD).
* Significant side-related differences of vowels (natural and random) pronunciation, three-way ANOVA.

Table 10. Maximum displacement of single landmarks (mm) during the execution of natural sequence vowels: mean ± SD values in 10 women and in 10 men. (R –right; L – left; ng – naso-genian; *ch* – cheilion; *cph* – crista philtri; *li* - lower lip midpoints).

	Natural sequence vowels																			
Landmarks (mm)	**a**				**e**				**i**				**o**				**u**			
	Women		*Men*		*Women*		*Men*		*Women*		*Men*		*Women*		*Men*		*Women*		*Men*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ng R	3.6	1.6	3.4	1.3	2.2	1.1	2.2	0.8	1.7	1.4	1.7	0.8	3.0	1.6	3.1	1.1	3.1	1.4	3.3	1.1
ch R	9.7*	2.7	7.6*	1.4	6.8*	2.4	4.8*	1.5	4.2	2.2	3.0	1.2	5.5	2.8	5.0	1.3	6.2	2.4	5.8	2.2
cph R	2.1	1.2	1.6	1.0	2.1	1.4	1.5	1.0	2.1	1.1	1.6	1.0	1.8	1.0	2.5	1.8	4.1	1.5	3.9	1.6
li R	19.7	6.9	17.6	2.2	12.9	5.3	11.4	3.8	8.7	2.3	9.2	3.2	12.4	4.2	12.7	3.2	7.4	1.9	9.0	2.2
ng L	3.8	1.5	3.1	0.9	2.3	1.4	1.9	0.8	1.7	1.3	1.7	0.6	2.7	1.2	3.2	1.4	2.8	1.4	3.1	2.1
ch L	9.3	3.3	7.2	1.5	6.9	3.3	4.4	1.6	4.1	2.2	3.6	1.2	5.4	2.2	6.1	3.0	5.5	2.2	6.2	4.5
cph L	2.0	1.3	1.7	1.4	1.8	1.2	1.5	1.1	1.7	0.9	1.8	1.2	1.4	0.7	2.7	2.2	3.5	1.5	4.2	2.0
li L	19.9	7.9	17.2	1.9	12.7	5.5	12.0	3.8	8.9	3.2	9.1	3.4	12.2	4.3	12.0	2.9	7.7	2.0	8.8	2.6

* Significant differences, Student's unpaired t-test (men vs women).

Table 11. Maximum displacement of single landmarks (mm) during the execution of random sequence vowels: mean ± SD values in 10 women and in 10 men. (R –right; L – left; ng – naso-genian; *ch* – cheilion; *cph* – crista philtri; *li* - lower lip midpoints).

Landmarks (mm)	**Random sequence vowels**																			
	a				**e**				**i**				**o**				**u**			
	Women		**Men**		**Women**		**Men**		**Women**		**Men**		**Women**		**Men**		**Women**		**Men**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ng R	3.4	1.4	2.9	1.4	2.0	1.2	2.0	0.6	1.9	1.1	1.9	1.1	3.3	1.1	3.4	1.7	3.0	1.2	2.9	1.3
ch R	7.8	1.8	6.9	1.7	6.7	3.7	5.3	2.0	5.0	3.1	4.1	2.5	5.9	1.7	5.2	2.1	6.1	2.1	5.6	1.8
cph R	2.1	1.5	1.5	0.8	2.6	2.3	1.9	1.4	2.6	2.8	2.4	1.3	1.9	0.7	2.4	0.9	3.3	0.7	3.2	1.9
li R	16.6	4.2	15.8	3.2	11.8	4.3	12.7	3.1	8.9	2.4	10.3	4.6	12.9	3.4	13.4	4.6	8.8	2.4	9.0	2.7
ng L	3.0	0.9	2.6	1.0	2.2	1.0	1.8	0.6	1.7	1.0	1.6	1.1	2.8	0.6	2.9	1.2	2.8	1.0	2.6	0.9
ch L	7.6	2.4	6.4	1.3	6.0	2.9	5.1	2.1	4.4	2.5	4.2	2.5	6.2	1.4	5.0	1.8	6.5	2.0	5.7	2.2
cph L	1.3	0.4	1.8	1.1	1.7	1.1	1.8	1.4	2.0	1.6	2.3	1.2	1.5*	0.9	2.8*	1.3	3.4	1.2	3.3	1.9
li L	16.6	4.3	15.5	3.2	11.5	4.3	12.2	3.2	9.0	2.7	9.9	4.8	13.3	3.3	12.5	4.3	8.5	2.4	8.6	2.1

* Significant differences, Student's unpaired t-test (men vs women).

Mouth asymmetry indices were not significantly different between sexes in both sequences of vowels (a, i) (Student's unpaired t-test; p-values < 0.05). On the other hand, in vowels pronunciation in natural sequence, significant differences were found in (li) landmark for vowel (e) ($p = 0.046$) and in (cph) landmark for vowel (u). In the random sequence vowel pronunciation, a significant difference was found in (cph) and (li) landmarks for vowel (o) ($p = 0.007$; $p = 0.017$) (Tables 12, 13).

For both sequences of vowel pronunciation, some significant differences from 0 were found. For the natural sequence, vowels e (li, $p = 0.046$) and u (cph, $p = 0.039$) were significantly asymmetric in women, and vowels a (total, $p = 0.024$), e (li, $p = 0.021$ and total, $p = 0.004$) and o (li, $p = 0.039$) in men. For the random sequence, a significant difference was found in women for vowels a (total, $p = 0.006$), e (cph, $p = 0.031$ and total $p = 0.025$) and o (cph, $p = 0.036$), and in men for vowels e (li, $p = 0.015$) and o (cph, $p = 0.020$ and li, $p = 0.042$).

Table 12. Mouth asymmetry indices for natural sequence of vowels in women and men (ch – cheilion; cph – crista philtri; li - lower lip midpoints).

%	Asymmetry (ch)		Asymmetry (cph)		Asymmetry (li)		Total Asymmetry	
women	mean	SD	mean	SD	mean	SD	mean	SD
a	3.5	8.3	4.6	27.9	0.0	2.3	1.1	4.1
e	2.6	10.8	0.9	29.2	-0.8*#	4.1	0.7	4.9
i	0.3	7.9	8.7	26.1	0.1	5.5	1.5	4.5
o	-1.7	14.5	10.2	16.6	0.6	3.2	1.3	5.0
u	6.8	10.8	9.7*#	12.9	-1.9	6.3	4.1	6.7
men	mean	SD	mean	SD	mean	SD	mean	SD
a	3.0	5.2	-2.6	23.7	1.1	2.2	1.8#	2.0
e	5.4	9.2	2.9	13.5	2.8*#	3.2	3.4#	2.6
i	-9.8	15.8	-3.1	26.4	1.9	5.2	-1.8	6.7
o	-5.9	16.5	-0.4	9.0	2.6#	3.6	-0.5	7.0
u	3.1	22.5	-2.5*	7.0	2.1	5.7	1.2	9.4

* Significant differences, Student's unpaired t-test (men vs women).
# Significant differences, Student's paired t-test (difference from 0).

Table 13. Mouth asymmetry indices for random sequence of vowels in women and men (*ch* – cheilion; *cph* – crista philtri; *li* - lower lip midpoints).

%	Asymmetry (ch)		Asymmetry (cph)		Asymmetry (li)		Total Asymmetry	
women	Mean	SD	mean	SD	mean	SD	mean	SD
a	2.9	7.0	13.4	28.5	0.1	2.0	$2.7^{\#}$	2.3
e	5.6	8.2	$19.8^{\#}$	24.6	1.5	3.9	$5.2^{\#}$	6.2
i	5.6	10.5	5.7	14.3	-0.4	3.7	3.3	6.3
o	-3.9	9.4	$18.4^{*\#}$	23.6	-2.1^{*}	4.7	0.1	4.1
u	-3.7	7.7	-0.2	11.2	1.5	6.3	0.3	5.4
men	Mean	SD	mean	SD	mean	SD	mean	SD
a	3.7	7.1	-6.9	23.5	0.9	2.1	1.4	3.0
e	2.5	13.8	4.0	27.2	$2.4^{\#}$	2.6	3.5	6.9
i	-0.8	15.2	-5.2	18.4	2.8	5.4	1.9	6.6
o	0.6	9.6	$-7.7^{*\#}$	8.5	$3.3^{*\#}$	4.5	2.0	4.8
u	0.1	9.2	-1.8	10.8	1.9	5.0	1.5	5.3

* Significant differences, Student's unpaired t-test (men vs women).
# Significant differences, Student's paired t-test (difference from 0).

On average, the ASYM index of the sequence of numbers and words pronunciation was similar in both sexes, without significant differences ($p > 0.05$, two-way factorial analysis of variance) (Figs. 22, 23).

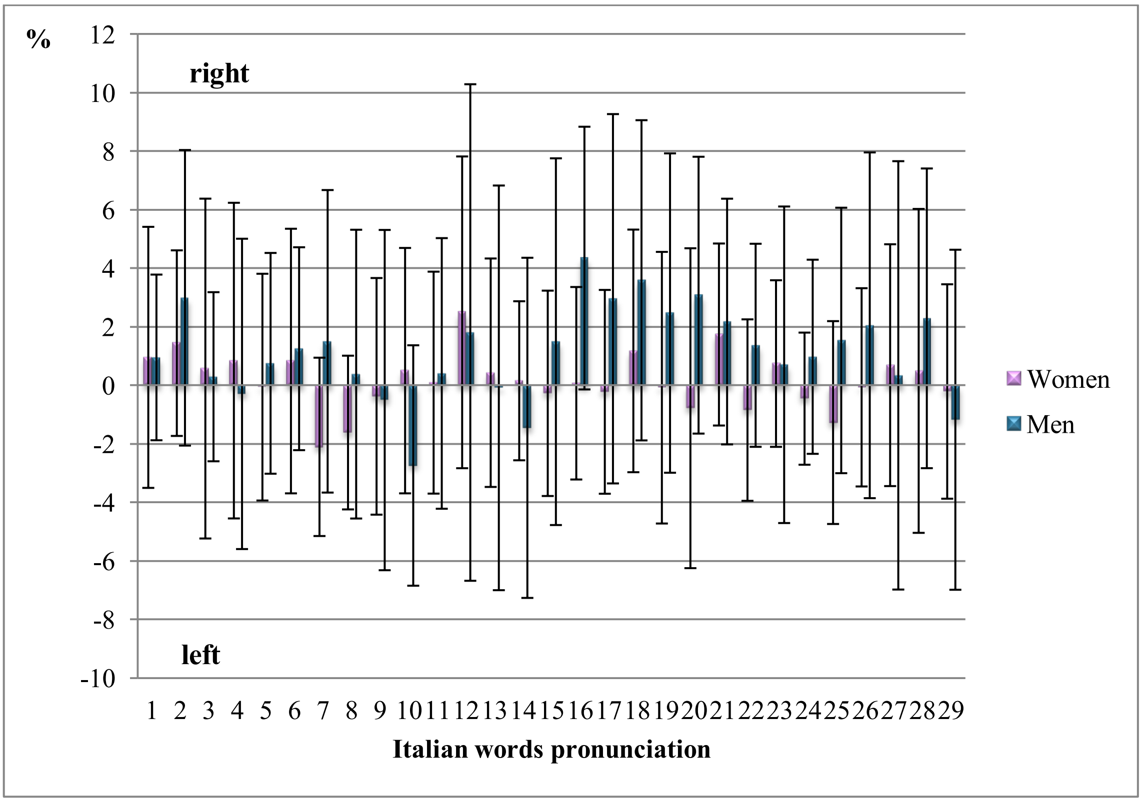

Figure 22. ASYM index in Italian words pronunciation (mean ± 1SD).

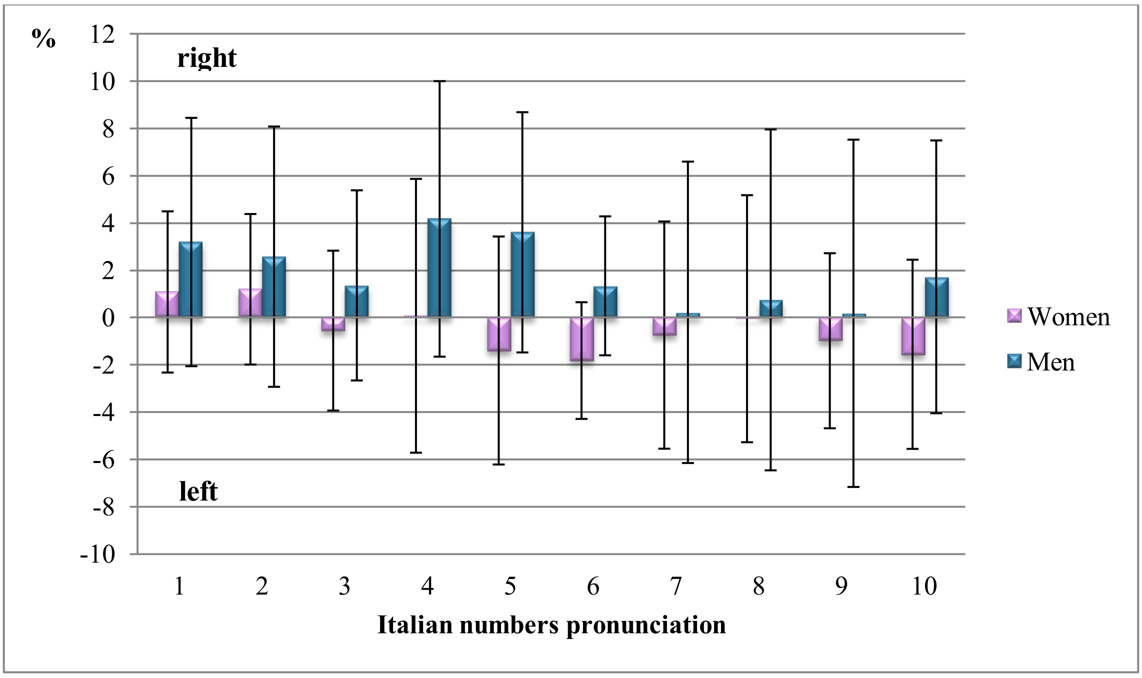

Figure 23. ASYM index in numbers pronunciation (mean ±1SD).

On average, the POC index for Italian words and sequence of numbers ranged between 91 and 96%, with significant differences in speech pronunciation ($p = 0.031$, two-way factorial analysis of variance; factors: sexes and speech pronunciation).

8 ELECTROMYOGRAPHIC ANALYSIS - HEALTHY SUBJECTS

8.1 METHODS

8.1.1 DATA COLLECTION

EMG indices for verbal and non-verbal activities are summarized in table 14. Data of 2 men and 2 women were lost for technical reasons in closed-mouth smile. In spontaneous smile, we analyzed only 15 subjects, because one subject did not perform the spontaneous smiles and data of other four subjects were lost for technical reasons.

Table 14. EMG indices for verbal and non-verbal activities (*r:* right, *l:* left).

	EMG INDICES			
	Asymmetry (*r* and *l* zygomaticus major)	Asymmetry (*r* and *l* depressor labii inferioris)	Asynchrony (*r* zygomaticus major and *r* depressor labii inferioris)	Asynchrony (*l* zygomaticus major and *l* depressor labii inferioris)
Open-mouth smile	✓	✓	✓	✓
Closed-mouth smile	✓	✓	✓	✓
Spontaneous smile	✓	✓	✓	✓
Natural numbers		✓		
Italian words		✓		

8.1.2 STATISTICAL ANALYSIS

For all subjects, ten series of open and closed-mouth smiles, spontaneous smile, natural sequence of numbers and words pronunciation were made. Descriptive statistics were obtained for electromyographic time onset in open-mouth, closed-mouth and spontaneous smiles, natural sequence of numbers, and words pronunciation.

The normal distribution of data was checked with the Kolmogorov-Smirnov test.

Both instructed smiles (open and closed-mouth), were compared by 4-way analyses of variance (factors: sexes; muscles; side; subjects). The spontaneous smile was compared by two-way analysis of variance (factors muscle, side, and muscle × side interaction).

The natural sequence of numbers and words pronunciations was compared by two-way analysis of variance (factors sexes and pronunciation).

The significance level was set at 5% for all statistical analyses ($p < 0.05$).

8.2 RESULTS

All data within each subgroup (men, women) were normally distributed (Kolmogorov–Smirnov tests, $p > 0.05$), and no significant difference was found between male and female mean ages (Student's unpaired t-test; all p-values > 0.05).

Significant interactions were found in muscle × subject in open ($p < 0.001$) and closed-mouth smile ($p < 0.001$; four-way factorial analysis of variance).

Overall, for both smiles the zygomatic muscles tend to anticipate the depressor labii inferioris muscles in women, while in men the opposite was found (Tables 15, 16).

Table 15. Onset timing of muscle activity in closed-mouth smiles.

Onset time				
	women		*men*	
Closed-mouth smile (s)	mean	SD	mean	SD
right zygomatic major	0.05	0.07	0.06	0.10
left zygomatic major	0.04	0.07	0.05	0.10
right depressor labii inferioris	0.07	0.07	0.06	0.06
left depressor labii inferioris	0.07	0.09	0.05	0.05

Table 16. Onset timing of muscle activity in open-mouth smiled.

Onset time				
	women		*men*	
Open-mouth smile (s)	mean	SD	mean	SD
right zygomatic major	0.06	0.07	0.07	0.07
left zygomatic major	0.07	0.07	0.06	0.07
right depressor labii inferioris	0.06	0.06	0.03	0.06
left depressor labii inferioris	0.07	0.07	0.04	0.05

In spontaneous smiles no differences were found (Table 17, two-way factorial analysis of variance; factors muscle, side and muscle × side interaction: all p-values > 0.05).

Table 17. Onset timing of muscle activity in spontaneous smiles.

	Onset time				
	right		*left*		ANOVA 2 ways
Spontaneous smile (s)	mean	SD	mean	SD	
zygomatic major	0.10	0.12	0.13	0.12	NS
depressor labii inferioris	0.09	0.11	0.10	0.11	NS

NS, not significant, p > 0.05.

For speech pronunciation, the onset time of activation was calculated only by the asymmetry between the right and left depressor labii inferioris muscles. No significant differences were found in both sexes and in speech pronunciation (two-way factorial analysis of variance; factor sexes, factor pronunciation: all p-values > 0.05).

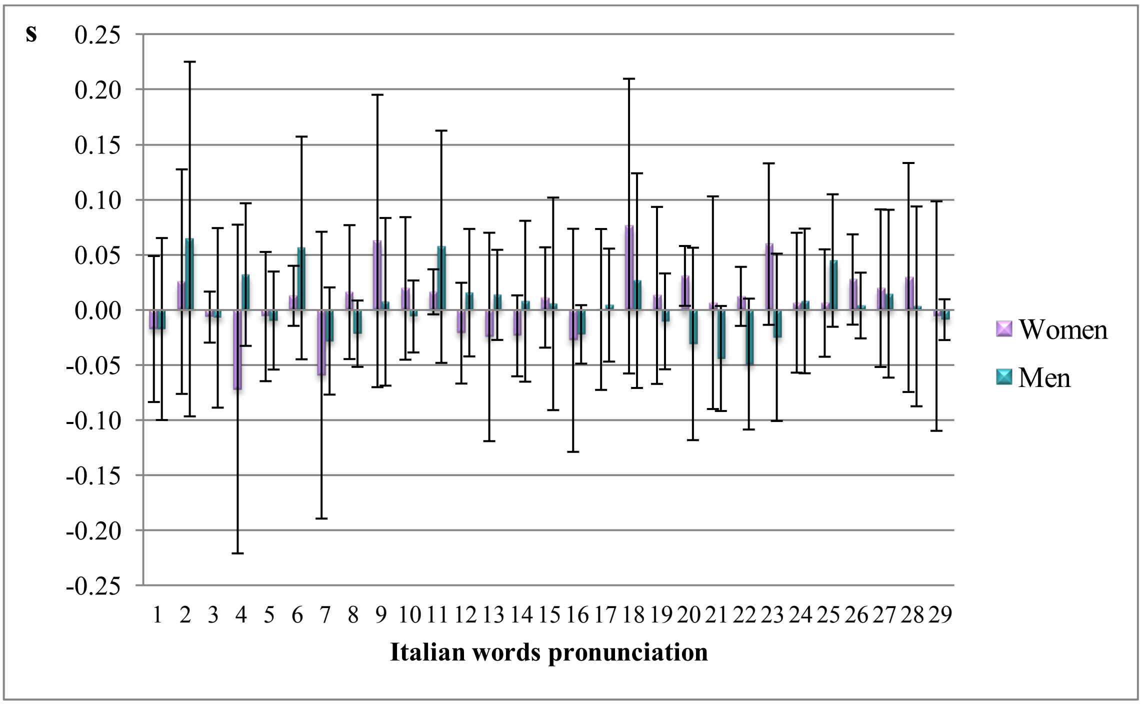

Figure 24. Asymmetry between right and left depressor labii inferioris muscles in both sexes (Italian words pronunciation) (mean ±1SD).

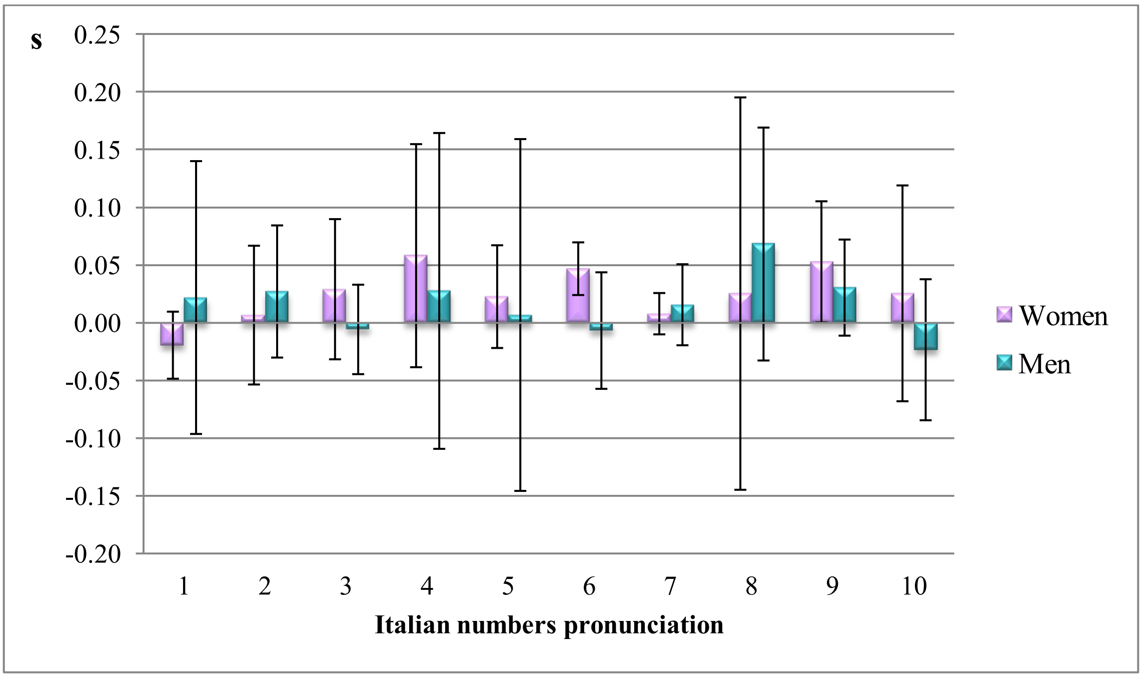

Figure 25. Asymmetry between right and left depressor labii inferioris muscles in both sexes (Italian numbers pronunciation) (mean ±1SD).

9 DISCUSSION

Emotions play a key role in social communication, and interactions in humans crucially depend on facial expressions. Spontaneous facial mimicry provides unique insights into the biological determinants of emotional responses. The neurological control of a smile consists of a complex process involving many facets, central pathways of voluntary and evoked smiling (Root and Stephens, 2003; Achaibou et al., 2008; Rymarczyk et al., 2011).

Facial expressions and speech articulation are produced by the synergistic or co-operative action of many different facial muscles, involving movements of the mouth (lower and upper lips) (Root and Stephens, 2003; Holberg et al., 2006; Van der Geld et al., 2008; Sjögreen et al., 2011).

The smile is characterized by an upward curving of the corners of the mouth, indicating pleasure, amusement, or derision, a pleasant or favorable disposition or aspect, which is produced by the contraction of the zygomatic major muscle. Zygomatic minor muscle (drawing the skin of the lip between the philtrum and the lip corner obliquely upwards and laterally) and levator Anguli Oris (incidentally displaying the teeth) also contributes to smiling.

According to Frank and Ekman (1993), each smile, using a combination of different muscles, conveys different messages, for example the spontaneous smiles are formed by the contraction of both the zygomatic major and the orbicularis oculi muscles, while posed smiles involve only the zygomatic major muscle. These different types of smiles are often distinguishable during social interaction.

Facial movements reflect spontaneous responses of viewers to emotional stimuli, and the identification of the neural correlates of this phenomenon can supply important findings about the brain mechanisms of emotion and social cognition. However, little is known about the neural processes underlying facial mimicry, and in particular whether emotional face perception and the subsequent emotion reaction are somehow related or completely independent processes (Achaibou et al., 2008).

The instruments

Facial mimicry can be assessed in two ways: by analyzing the effectors of the movements (that is, by surface or needle EMG of facial muscles) or by investigating the final result of muscle contraction (that is, by three-dimensional motion analysis of selected facial landmarks). Both methods have advantages and limitations.

Several instruments have been developed for the non-invasive assessment of facial movements; in particular, optoelectronic motion analyzers working with passive, retroreflective markers appear to be the best suitable for data collection in both patients and healthy subjects. These instruments allow a complete and detailed assessment of motion in all parts of the face collecting both qualitative and quantitative data (Trotman et al., 1998a,b; Weeden et al., 2001; Coulson et al., 2002; Johnston et al., 2003; Mishima et al., 2004; Nooreyazdan et al., 2004; Ferrario and Sforza, 2007; Agostino et al., 2008; Sforza et al., 2010b,c; 2012; Popat et al., 2010; Verzé et al., 2011a,b).

The optoelectronic instrument used in this study was calibrated with an accuracy lower than 0.02%. This means that the movement of each 2-mm marker could be detected within 0.12 mm, a value similar (Hontanilla and Aubá, 2008), or even better (Trotman et al., 1998a, 2003) than those reported in previous studies.

While motion analysis inform only about the amount and quality of the movements, EMG can offer more insight into the neuromuscular determinants of the action. In particular, needle EMG is used mainly in clinics for the detection of the activity of selected parts of the muscles, and its applications in research are limited by its invasiveness. In contrast, surface EMG provides non-invasive data that can be easily obtained also in control subjects without side effects, discomfort or pain. A limitation of surface EMG is the electrode dimension: small muscles and single muscular bellies or bundles cannot be investigated by themselves, and all recordings will be contaminated by cross-talk.

In the current study, great care was taken to ensure the best positioning of the electrodes, limiting the occurrence of cross-talk. Additionally, surface EMG is vulnerable to extra-muscular factors that may alter and distort the true electric signal. One of the principal problems that limit a widespread use of surface EMG in clinics is the necessary normalization of its recordings, to reduce the ‘biological noise’, and to allow useful comparisons between different subjects and different studies (DeLuca, 1997; Ferrario et al., 2000a).

Normalized data can inform the influence of various conditions on the neuromuscular activity, avoiding individual variability (anatomical variations, physiological and psychological status, etc.) and technical variations (muscle cross-talk, electrode position, etc.), toward the performance of clinically useful longitudinal and cross-sectional assessments (Ferrario et al., 2009).

Data normalization is a practice currently used for the assessment of other body muscles (DeLuca, 1997; Hagg et al., 2004). Indeed, to compare EMG recordings in the amplitude and frequency domains among different subjects it is necessary to relate all measurements to the electrical muscle activity detected during some standardization recording, like a maximum voluntary contraction (MVC) (Castroflorio et al., 2005, 2008; Cecílio et al., 2010; Forrester et al., 2010; Santana-Mora et al., 2009). EMG potentials collected in MVC have been reported to have the best repeatability (Suvinen et al., 2009; Forrester et al., 2010).

For the jaw elevator muscles, masseter and temporalis muscles, an MVC on cotton rolls has been reported to have the lowest inter-individual variability (Ferrario et al., 2006b; Felício et al., 2009b; Forrester et al., 2010), and a method based on this standardization has been in use in the last 10 years (Ferrario et al., 2000a; Tecco et al., 2011). Unfortunately, no protocol of this kind has been devised for mimetic muscles yet, and further investigations on this topic are necessary.

To avoid this limitation, we used only the time-related information provided by surface EMG, and analysed asymmetry and asynchrony in time and not in the amplitude domain of the EMG signal. Nonetheless, a large variability of EMG onset timing was found in this study. Part of the variability may derive from the measurement protocol. Indeed, EMG data were sampled with a 1 ms temporal resolution (1 kHz data sampling). Considering both signal noise, and that we used a semiautomatic method for the detection of the beginning of muscle activity, with some inter-observer variability, we decided to report data with a 10 ms resolution (0.01 s).

The movements

In the current investigation symmetric lip movements involving the lower facial third were analyzed. Lips movements and a speech pronunciation were performed with landmarks positioned around the mouth, as previously reported in literature (Holberg et al., 2006; Trotman et al., 1998a; Weeden et al., 2001; Sforza et al., 2010b,c; 2012). Data were collected in healthy subjects that performed rest/maximum lip movements (Weeden et al., 2001; Coulson et al., 2000; 2002; Hontanilla and Aubá, 2008; Sforza et al., 2010b,c; Sjögreen et al., 2011). Two categories of movements were analyzed: standardized smiles and lip purse, and speech (verbal) movements (Popat et al., 2008a,b; 2012; Vimercati et al., 2012; Sidequersky et al., 2012). In a recently study, Popat et al. (2010) reported better reproducibility for speech movements than for non-verbal ones. The use of standardized non-verbal movements should therefore always be coupled with more natural motions that may enhance individual performance (Holberg et al., 2006; Proff et al., 2006; Sforza et al., 2010b,c; 2012).

In this study the used words are composed by Italian language phonemes in various positions. Some of the word postures are bilabial speech that were used to give a good representation of lip movement, that were coupled with sequences of numbers and vowels, to better analyze the performance of lip movements.

In vowel (a) we observed the largest total mobility, but this movement is accomplished by both the temporomandibular joints movements and facial muscles. Previous investigations found that the largest labial movements involved open mouth animations (Ferrario and Sforza, 2007; Sforza et al., 2010c).

Sex differences

In spontaneous smile, a sex difference was found, showing larger displacement in men than in women. Previous studies reported significantly larger movements in men than in women (Giovanoli et al., 2003; Tzou et al. 2005; Sforza et al., 2010b), but those differences were explained by the larger male facial dimensions. Sforza et al. (2010b) used standardized movements and investigated actual proportional differences independently from facial dimensions. In the current study, men had a total lip frontal area larger than women. Similar anthropometric differences were recently reported in stereophotogrammetric studies (Ferrario et al., 2000b; Sforza et al., 2010a).

During non-verbal activities, the cheilion landmarks had the largest displacements in both sex groups, paralleling the results reported by Sforza et al. (2010c) in both young and mid-aged healthy adults of both sexes.

No sex-related differences were found in non-verbal movements, pronunciation of numbers and words sequence, whereas sex-related differences were found in vowels (e, o, u). In closed-mouth smile, a significant asymmetry in crista philtri landmark movements was found in women. In young healthy women, Sforza et al. (2010c) found a left-side prevalence in mouth asymmetry in the “surprise” animation, and during eye closing (cheilion and lower lip landmarks).

Asymmetry and asynchrony

In accord with the current findings, the movements were larger on the left than on the right side of the face during the execution of both instructed symmetric movement (Coulson et al., 2002; Giovanoli et al., 2003; Tzou et al., 2005), and emotional facial expressions (Borod et al., 1998; Nicholls et al., 2004; Okamoto et al., 2010), while in others studies the right-side movements were somewhat larger than the left-side ones in instructed symmetric movement (Ferrario et al., 1994, 1995, Shaner et al., 2000; Sforza et al., 2010c).

Some investigations explain the asymmetries found for facial expressions by the different information processing styles of two hemispheres: the right-side cerebral hemisphere exceeds the left-side one in processing emotional information, and it can control the left side of the face better (Borod et al., 1998, Nicholls et al., 2004; Okamoto et al, 2010; Sforza et al., 2010d). The different nervous supplies of the upper facial third (bilateral central nervous system commands to the facial nerve nuclei) and lower facial third (only commands to the contralateral nucleus) should also be considered (Urban et al., 2001; Ercan et al., 2008; Okamoto et al, 2010). Another hypothesis for larger left side facial movements is the influence of handedness (Coulson et al., 2002).

Some differences were found between the natural and random sequences of vowels pronunciation, with a somewhat larger asymmetry for random sequences. We tested both conditions to better describe the movements even in relatively “unusual” situations. Unfortunately, no previous study reported data on this topic, and further investigations are necessary.

In literature the influence of gender on hemispheric specialization for emotion was suggested, and some studies of emotional processing suggested that females show more lateralization than males (Ladavas et al., 1980 in Borod et al., 1998).

In the current study, no sides-related differences were found in EMG onset timing in smiles and speech movements. In literature, some studies have broadly investigated the asymmetry in facial expressions from the zygomatic muscle, and greater activity on the left than on the right side was reported (Dimberg and Petterson, 2000; Zhou and Hou, 2006). These investigations analyzed the strength of the EMG signal and not the timing of the onset. When measuring asymmetry and asynchrony in the strength of muscular action, both measures may be affected by anatomical factors independent on the strength of muscular contraction (e.g. thickness, mass and elasticity of the skin).

For open-mouth and closed-mouth smiles the zygomaticus major muscles tends to anticipate the depressor labii inferioris muscles in women, while in men the asynchrony between these muscles was opposite. The asynchrony differences in EMG onset timing may result from differences in the innervation and mechanical properties of upper and lower facial muscles.

The motoneurons pools controlling the muscles of facial expression reside in a brainstem nucleus called the facial motor nucleus. This nucleus lies in the caudal pontine tegmentum, and contains a musculotopic arrangement of subnuclei, each innervating a small group of facial muscles (Root and Stephens, 2003).

The upper facial muscles are bilaterally innervated, while the lower facial muscles are contralaterally innervated, with the largest group associated with the mouth (Urban et al., 2001; Ercan et al., 2008; Okamoto et al, 2010). Furthermore, the facial muscles groups are partially overlapping, and the cheek region represents a particularly crowded region. The zygomaticus major and depressor labii inferioris muscles lie in close proximity to a variety of muscle groups, thus being surface EMG recordings of these muscles groups is susceptible to cross talk (Larsen et al., 2003).

For speech pronunciation, the brain must generate motor commands to control the activation of many different motoneurons pools, which include those innervating the muscles of the articulators, the larynx, and the chest wall (Smith, 2006). The physiology of how the different facial nerve branches activate their target muscles when we smile is a mechanism to be explored. Within each facial nerve, motoneurons may fire at a different timing to produce a facial movement, such as a smile.

10 GENERAL CONCLUSIONS

The three-dimensional movement analysis is an important step in the description of facial function and morphology. In Speech-Language Therapy, nowadays, one of the greatest challenges is to quantify the results obtained by rehabilitation processes, associated to motor patterns and muscle contraction control.

Quantitative, objective and accurate evaluation of lips, facial muscle activity and speech pronunciation is essential for a better understanding of the normal function and for grading of facial dysfunction in patients during diagnosis, treatment and follow-up of their disorders.

Current clinical assessments and medical treatments are increasingly evidence-based, relying on a widespread diffusion of diagnostic tools and treatment protocols that should make scientific-based options available to the largest number of health professionals. In an effort to make diagnosis as objective as possible, a non-invasive protocol of involving both EMG signals and kinematic data was developed, in order to develop estimations of expressions and speech in clinically healthy subjects.

The outcomes suggest that the proposed method could be a useful tool to evaluate the patients with facial lesions. Assessment of these patients would profit from this quantitative approach, thus reducing the discordance among several clinical examinations.

The principal limitation of this study is the number of analysed subjects, which was under the proper sample size to avoid type II errors. Another limitation is the variability of the electromyography onset timing of the subjects in verbal and not verbal activities.

Additionally, a concurrent analyses of the EMG and landmark displacements signals is missing: we limited our present investigation to separate assessments, but a new data analysis protocol is currently under development, and these data will be re-analyzed trying to combine the information on the effector (muscular activation) and on the final effect (landmark displacement).

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