UNIVERSITA’ DEGLI STUDI DI MILANO

Phd in Oral Sciences-XIXX cycle

STANDARDIZED ELECTROMYOGRAPHIC ANALYSIS OF SWALLOWING AND CLINICAL APPLICATIONS.

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1. INTRODUCTION

1.1. Swallowing: a fundamental, but still not completely clear, function.

1.2. Physiology of swallowing

1.2.1. the oropharyngeal phase of swallowing

1.2.2. the esophageal phase of swallowing

1.3. Neuroanatomical basis of swallowing

1.3.1. sensory information

a. mechanoreceptors

b. proprioceptors

c. other receptors

1.3.2. afferent fibers

1.3.3. central pattern generator (CPG)

a. motoneurons

b. interneurons

1.3.4. neural network

1.3.5. superior swallowing control

1.3.6. neural mechanism

1.3.7. muscular coordination

1.4. Swallowing process evaluation

2. RESEARCH PROTOCOL

2.1. introduction and rationale of the study

2.2. material and methods

2.2.1. study design

2.2.2. subjects

2.2.3. instrumentation

2.2.4. measurements

2.2.5. sEMG data analysis

2.2.6. statistical evaluation

2.3. results

2.4. discussion

2.5. conclusions

3. REFERENCES
1. INTRODUCTION

1.1. Swallowing: a fundamental, but still not completely clear function

Swallowing is a complex physiological function which is repeated more than 2400 times every day. Its primary function is linked with the nutrition of the body. It involves many different anatomical structures, portions of the digestive and respiratory system, more than 25 pairs of muscles and different neurological pathways. All these parts have to be well coordinated in order to efficiently complete the swallowing process. The importance of the task is demonstrated by its first appearance in the normal human fetus by the 12th gestational week, before the cortical and subcortical structures have developed. The principal and obligate actors of the swallowing are led by automatic and reflex phenomena, which occur without voluntary interference and try to maintain the integrity of the sequence of movements. In fact swallowing can be triggered by nervous stimulation at different levels, without subjects consciousness. The main automatic events are the pharyngeal elevation and contraction, the nasal and laryngeal airway protection and finally the peristaltic esophageal wave. These events involve obligate swallowing muscles, such as submental, pharyngeal, laryngeal, esophageal muscles and the muscles of the soft palate. However, as it will be largely explained, during everyday life the automatic sequence of swallowing is anticipated by a first phase, which is under voluntary control and which can be identified as the oral phase. It mainly involves jaw elevator muscles, cheek and extrinsic tongue muscles, identified as facultative swallowing muscles. Swallowing problems or dysphagias are traditionally divided in oral preparatory, oral, pharyngeal and esophageal phase problems (van den Engel-Hoek et al 2017). Dysphagia is defined by medical dictionaries as difficulty in swallowing. The recognition of the different patterns of symptoms may aid in diagnosis and treatment (Carucci and Turner 2015). The videofluoroscopy is considered to be the gold standard for swallowing sequence evaluation, however to date a simple, non-invasive and reliable method in order to instrumentally assess the activation of the muscles of the masticatory system (jaw closing muscles and submental muscles) is still lacking.
Dentists have always been studying the physiological mandibular position and the best vertical dimension for each patient, during prosthetic rehabilitations. For many years the mandible stability during swallowing has been largely studied, since it was considered a physiological mandibular position. In addition due to the automatic nature of the task, the position of the mandible is repeatable (Campos et al 1996). However the swallowing without occlusal contact is a pattern often found in the population and not necessarily an incorrect swallowing pattern (Pernambuco et al 2011). Since a standardized protocol for the evaluation of the masticatory muscles contribution during swallowing is still lacking, we decided to develop a method applied on a “controlled” kind of swallowing, asking the subjects to keep their teeth in contact during the function. Thus a repeatability of the mandible position and a muscular recruitment model may be drawn, even though the requested task is a not fully physiological one. Once the protocol will be developed, it will be applied to a “more physiological” kind of swallowing, asking the subjects to swallow in their most comfortable way, without giving them instructions about teeth position. Thus speculations about the activation and the symmetry of masticatory muscles during the oral phase of swallowing in healthy subjects can be done. In the future the method could be applied on different clinical situations (atypical swallowing, dysphagia, patients undergoing prosthetic rehabilitation) in order to compare their muscular pattern, to find possible alterations aiming at the optimization of the treatment, by a personalized measure of the function.

1.2. Physiology of swallowing

The digestive system transports food internally from the environment to a tissue interface for nutrients to reach cellular components of the biological system. The passage of the bolus from the mouth to the esophagus requires the interaction of both the digestive and respiratory tracts, with swallow providing the propulsion of the food from the oral cavity into the stomach, as well as providing a protective reflex for the upper respiratory tract (Lavelle 1988). Swallowing is a complex sensorimotor behaviour involving the coordinated contraction and inhibition of the musculature located around the mouth and at the tongue, larynx, pharynx and esophagus bilaterally (Ertekin and Aydogdu 2003). It has become convenient to state that swallowing is subdivided into three phases: oral, pharyngeal, and esophageal. This conventional division of the human
swallowing is usually ascribed to Magendie (1825) (Miller 1982). The swallow has, however, also been described in two stages i.e. the buccopharyngeal (or oropharyngeal) and esophageal stages (Jean 2001). The 3 phases of swallowing are probably related to their innervation pattern: the oral phase is often accepted as voluntary, while the pharyngeal phase is considered a reflex response and the esophageal phase is mainly under dual control of the somatic and autonomic nervous systems (Miller 1982). Since the oral and the pharyngeal phases are anatomically and functionally linked, an oropharyngeal phase will be considered. In fact oral, pharyngeal and laryngeal muscles co-work throughout the whole task.

1.2.1. Oropharyngeal phase of swallowing

The duration of the whole oropharyngeal sequence is found to be in the range of 0.6–1 s and remarkably constant in all the mammals studied, including humans (Jean 2001). The oral moment of swallowing is mainly voluntary and highly variable in duration depending upon taste, environment, hunger, motivation and consciousness for the human subject. Its primary function is the movement of the tongue, pressing the bolus against the hard palate and initiating the movement of bolus to the posterior part of the tongue and toward the oropharynx. The submental muscles (floor of the mouth) are particularly important to elevate the tongue, especially for solid bolus. In this stage, the contraction of the lips and cheek muscles (i.e. orbicularis oris and buccinator muscles) are crucial to prevent the escape of solid or liquid from the oral cavity (activation of the VII cranial nerve) (Ertekin and Aydogdy 2003). After the mastication of a solid bolus, or the intake of a liquid, the dorsal portion of the tongue forms a spoon-shaped depression in the anterior midline. The anterior half of the tongue is then pressed against the maxillary alveolar ridge and the anterior part of the hard palate in a rapid sequence; moving the bolus posteriorly on the root of the tongue toward the pillars of the fauces (Lavelle 1988). This stage is ended by the triggering of the pharyngeal phase of swallowing. The nature of the triggering of the pharyngeal phase of swallowing is not clearly known. As it will be largely explained the afferent fibers involved in the initiation of swallowing are those running within the trigeminal nerve, the glossopharyngeal nerve and the vagus nerve, especially its superior laryngeal branch. In healthy human subjects, it is evident that there is usually a gradual accumulation of
prepared food on the posterior surface of tongue and this solid food reaches the glossoepiglottic vallecula before the initiation of the swallow. In a small volume swallow (1–2 ml) such as saliva, there is no oral preparation and the oral and pharyngeal stages occur in sequence. In contrast, when taking a large volume liquid bolus, the oral and pharyngeal stages overlap with each other, occurring simultaneously. The size of the bolus does not alter the sequence of events during oropharyngeal swallowing but modulates the timing of each phase of the swallow. As the bolus size increases (1–20 ml), the pharyngeal transit time increases, as it does laryngeal closure. Although the site, timing and intensity of the oropharyngeal sensory input may vary between different bolus and between healthy subjects and patients with sensory impairment, once swallowing is initiated, the cascade of the sequential muscle activation does not essentially alter from the perioral muscles downward. This is one of the lines of evidence for the existence of the central pattern generator (CPG) for the human swallowing that will be discussed later (Ertekin and Aydogdu 2003). The oral stage is subjected to marked inter-individual variability, whereas the pharyngeal stage is generally consistent (Lavelle 1988). When the movement of the bolus from the oral cavity to the pharyngeal spaces triggers the swallowing reflex or response, the following physiological events occur in rapid overlapping sequence. These events are as follows (Ertekin and Aydogdu 2003):

- The nasal, laryngeal and tracheal airway is protected by several “reflex” events including the closure of the velopharyngeal isthmus by the palate, laryngeal elevation and suspension by suprahypoid/submental muscles and closure of the larynx by laryngeal muscles of the vocal folds and epiglottis. Laryngeal elevation is a vital component of the airway protection as this action does not only facilitate closure of the vestibule but also repositioning of the larynx anterosuperiorly under the tongue base. All swallows take place somewhere between late inspiration and late expiration, and there is always an apneic period during the pharyngeal phase of swallowing.
- The tongue thrusts posteriorly to push the bolus throughout the pharynx and into the esophagus (XII cranial nerve). A sequential wave of contraction of the pharyngeal constrictor muscles (X cranial nerve) clears any remaining material into the esophagus. The main propulsive force acting on the bolus is thus,
provided by the posterior movement of the tongue. The pharyngeal contraction seems to be minimal in relation to bolus propulsion, although it facilitates subsequent pharyngeal clearance in association with a profound shortening of the pharynx. Simultaneously the larynx rises and it is pulled under the root of the tongue and the epiglottis folds down over the laryngeal opening. During this phase of pharyngeal constriction, the epiglottis tips inferoposteriorly and the true and false vocal cords protect the laryngeal vestibule by constricting the laryngeal aperture. Thus the epiglottis facilitates passage of the bolus through the piriform fossae and into the esophagus (Lavelle 1988).

- The upper esophageal sphincter (UES) relaxes and opens for the bolus transport into the esophagus. The UES consists primarily of the tonically contracting striated cricopharyngeus muscle. During a swallow, this muscle relaxes and is opened and the sphincter is pulled upon anteriorly by the contraction of the suprahyoid/submental muscle groups. Then the pharyngeal phase of swallowing is completed and the UES closes until the next swallow. The cessation of tonic activity of the CP muscle is likely believed to be due to a neural inhibition, possibly originating from the CPG at the medullary level.

It is believed that the maxillary and mandibular teeth usually make contact during swallowing. This tooth contact is considered to stabilize the mandible while the hyoid and larynx execute superior-anterior movements (Lavelle 1988). Nevertheless this issue is still controversial because tooth contact, lip and tongue movements, vary considerably between patients.

1.2.2. The esophageal phase of swallowing

In comparison with the extraordinary complexity and rapidity of the oropharyngeal phase, the esophageal phase of swallowing is simpler and slower. It consists of a peristaltic wave of contraction of the striated and smooth muscles, which propagates to the stomach. The peristaltic contraction moves from the proximal to the distal part of the esophagus at a speed that may show a fairly high degree of variability, depending on the species and the nature of the muscles, i.e., on whether an esophagus is composed of striated muscle alone or of both striated and smooth muscle (Jean 2001) (Fig 1). This peristaltic sequence of the esophagus during swallowing, termed primary peristalsis, is
distinct from secondary peristalsis, which is initiated by distension as a bolus is placed directly within the esophagus (Lavelle 1988). Secondary peristalsis occurs in response to stimulation of sensory receptors in the esophagus. For example, the transient esophageal distension induced by rapidly inflating an intraluminal balloon can induce peristaltic contractions in the esophagus. This may correspond to the distensions produced when the esophageal content is not completely cleared by the first swallow, or when reflux of the gastric contents occurs into the esophagus. Secondary peristalsis may be initiated at the level of either the striated or the smooth muscle. The wave of contraction usually begins at the level of the distension or just above it (Jean 2001).

Figure 1: Lateral view of a swallow in a healthy person, based on videofluorographic recordings. (A) Food (shown in green) is sitting on the dorsum of the tongue. A portion of food is already in the valleculae, having been propelled there during a previous oral propulsive cycle. (B) Moving upward and forward, the tip of the tongue comes into contact with the hard palate anteriorly. (C) The area of tongue-palate contact expands posteriorly, which pushes additional food into the oropharynx. The soft palate and larynx begin to elevate and the epiglottis begins to tilt. (D) Pushing back into the pharynx, the tongue squeezes the bolus downward through the hypopharynx. The hyoid bone and larynx are pulled upward and forward; as a result, the upper esophageal sphincter opens. (E) The tongue continues pushing backward, and the bolus passes through the upper esophageal sphincter. The posterior pharyngeal wall pushes forward to come into contact with the posterior surface of the tongue. This clears the pharynx of residue. (F) The tongue drops away from the palate, the larynx and nasopharynx open, and the upper esophageal sphincter closes as the bolus passes down the esophagus (Palmer et al 2000).
1.3. Neuroanatomical basis of swallowing

Swallowing is a well organized and coordinated task in which the cerebral cortex, motor nuclei of the brain stem and the sensori-motor fibers play a crucial role. Swallowing is usually thought to be the result of local peristaltic mechanisms in the esophagus, combined with reflex involvement of swallowing centers in the brainstem. However, the cerebral cortex appears to play a critical role in the initiation of voluntary swallows. Indeed, repetitive electrical stimulation of appropriate regions of the cortex in anaesthetized animals or in humans undergoing neurological surgery can induce swallowing (Hamdy et al 2000). The precentral gyrus of the frontal lobe takes part in the first and voluntary part of swallowing (Hamdy et al 2000). The central pattern generator (CPG), the premotor circuitry and the motor neurons controlling the phases of swallowing are contained in the brain stem (Lang 2009). Corticobulbar fibers connect the cortex with those subcortical centers for the completion and the regulation of the tasks. The knowledge of mechanism and regulation of swallowing has a crucial role both in research and clinics.

1.3.1. Sensory information

The sensory information plays an important role in swallowing regulation, in fact the oro-pharyngeal-laryngeal area shows an high concentration of receptors. Sensory input not only has a major influence on the activity of brainstem swallowing centers but also converges onto cortical sensory and motor areas. Furthermore, it has been shown that the excitability of cortical projections to swallowing muscles can be influenced by the stimulation of afferent fibers in the vagal and trigeminal nerves (Hamdy et al 2001). The role of sensation in swallowing has been the subject of controversy over the years. As an example during dental anesthetic the oral phase of swallowing can be altered. We also know that salivary flow and that other autonomic events depend on the stimulation of sensory receptors (Corbin-Lewis and Liss 2005). In addition olfactory and visual afferences co-work for the anticipatory phase of swallowing. Sensory inputs from peripheral areas play an essential part in inducing the whole swallowing motor sequence or parts of this motor sequence such as esophageal peristalsis. They play the prime role in reflex swallowing and they are also involved in voluntary swallowing. Chemical or mechanical stimuli can activate reflex pharyngeal swallows, indicating that activation of either chemo- or mechanoreceptors may be effective, but the specific role is still
unknown. Studies have found that the most sensitive pharyngeal site for activation of swallowing due to focal pressure is the anterior hypopharynx, but the larynx is more sensitive than the pharynx to either chemical or mechanical stimulation (Lang 2009). Moreover, sensory inputs modulate the central network activity to adapt the forthcoming motor sequence to the information arising from peripheral receptors. Although the swallowing motor sequence is centrally organized, it can change as the result of peripheral afferent information (Jean 2001). Receptors turn physical or chemical inputs into nervous impulses. The mouth has a special status within the somato-sensory system. Firstly, it is one of the most densely innervated part of the body, in terms of receptors. This sensory richness is linked to the key role of oral sensorimotor control in eating, drinking and speaking, as well as to the vivid nature of many oral sensations. Secondly, the mouth contains a large range of different tissue types (skin, muscle, teeth) in close proximity and constant interaction. These generate very rich patterns of somato-sensory afferent input. Thirdly, being a cavity, it has some somato-sensory properties typical of the external surfaces of the body, and others more characteristic of the internal milieu. Thus, oral sensations provide an important interface experience, of both the objects in the mouth and of the states and movements of the mouth itself. Nevertheless, oral somato-sensation remains relatively little understood (Haggard and de Boer 2014). A study conducted by Longo et al in 2010 showed an interesting model of the oral somato-sensory awareness. The model presents a hierarchy of three stages of sensory processing, reflecting identified levels in the somato-sensory pathway. The first level is somato-sensation proper. This refers to the awareness of individual afferent events, such as touches, noxious stimuli, etc. The second level, which we call somato-perception, refers to the processing of several sensory inputs to form a percept of a specific object or stimulus source. A crucial feature of this level is the integration and combination of information from different receptor types and different regions of the receptor surface. The third and final level of the somato-sensory hierarchy is somato-representation. This refers to the representation of the body as an object in itself. Through continued somato-sensory and other inputs, we gradually build a representation of what our body is like, i.e., a conscious image of the body as a physical object. Importantly, this representation cannot be generated directly by any single somato-sensory afferent signal (Longo and Haggard 2010). The three processing
stages of the theoretical model shown in Fig 2 can be related to different stages of the oral somato-sensory pathway (Haggard and de Boer 2014).

![Figure 2: different stages of the oral somato-sensory pathway (Haggard and de Boer 2014).](image)

a. **Mechanoreceptors**

Mechanoreceptors fire when they are mechanically deformed and their discharged frequencies is related to their deformation and thus to the applied pressure. With regard to swallowing, this may result from mucosal contact with the bolus or by its contact with other oral structures during mastication (Corbin-Lewis and Liss 2005). Together with chemical receptors, they are the most represented in the oral cavity and their characteristics change depending on their position. They can be found in the oral mucosa (tongue, gingiva, palatal mucosa, vestibular and malar mucosa), in the temporomandibular joint (capsule and disc) and in the periodontal ligament. The structures that mediate mechanosensation in the mouth include Merkel cell complexes, Ruffini type endings, Meissner endings and Pacinian corpuscles. Mechanoreceptors of the anterior region (i.e. tongue tip) have a high discriminative capacity, in order to well understand food shape and dimension (oral stereognosis); this capacity becomes less and less important going back to the tongue. Important receptors can be found also in the pharyngeal and laryngeal area and in the periodontal tissues, which give information about the hardness of the food and so about the muscular strength required for its mastication. The periodontal mechanoreceptors provide important information regarding temporal, spatial and intensive aspects of force acting on the tooth, during the initial tooth food contact (Kumar et al 2016). Behavior of receptors depends on their
localization. They are mostly located in the apical third of the ligament. The most rare and rapidly adapting receptors are situated below but closer to the fulcrum than to the apex (Turker 2002). Periodontal mechanoreceptors respond maximally when the area in which they lie is put into tension (i.e. stretch). The receptor types found in the TMJ capsule include free nerve endings, Ruffini endings, Golgi organs and Pacini corpuscles. It has been claimed that the Ruffini endings and the Golgi organ within the capsule function as static mechanoreceptors, the Pacini endings as dynamic mechanoreceptors and the free nerve endings as the pain receptors (Turker 2002). Touch and pressure have been used to stimulate pharyngeal swallowing in human subjects and experimental animals. Larger bolus volumes elicit greater tongue propulsive forces and shorter latencies to evoke the swallow. Another bolus characteristic detected via touch and pressure mechanoreception is viscosity. Higher bolus viscosities elicit increases in oropharyngeal transit times, intrabolus pressures, duration of pharyngeal peristalsis, duration of tongue base contact to the posterior pharyngeal wall, duration and excursion of hyoid movement and duration of upper esophageal sphincter relaxation and opening. Furthermore the ingestion of solid foods involves transport of the bolus to the occlusal surface of the molar teeth where the bolus is reduced to smaller-size pieces and then transported into the vallecular space before falling into the pharynx. This pattern of ingestion contrasts with that usually observed in single (discrete) sips of liquid, in which the bolus is held in a chamber between the dorsal surface of the tongue and the hard palate and then squeezed in a rostrocaudal direction toward the pharynx by virtue of upward and anteriorly directed tongue movements. Discrete boluses of liquid do not usually accumulate in the hypopharynx prior to swallow onset, except in the case of sequential liquid swallowing and during straw drinking (Steele and Miller 2010).

b. Proprioreceptors

Kinesthetic receptors are related to swallowing too. Proprioceptors are located in the muscular fibers (neuromuscular spindles MS) and in the tendons (Golgi tendon organs). Both of them are crucial in maintaining muscle tone and facilitating controlled movements.
The spindles in skeletal muscles give information about muscle length and they are part of a complex functional system. They possess multiple roles such as generating antigravity thrust during quiet upright stance, timing of locomotor phases, correcting for muscle nonlinearities, compensating muscle fatigue, determining synergy formation and modulating plasticity. The number of MS in a muscle seems to be related to its function and widely varies from one muscle to the other. Muscles active in gross movements have low spindle density whereas muscles initiating fine movements or maintaining postural stability have a high spindle density (Osterlund et al 2011). In humans, masticatory muscles (jaw-closing muscles and jaw-opening ones) have different concentration of MS. The adult human masseter muscle contains especially large and complexly arranged muscle spindles. Typical features are a high muscle spindle density, large capsule diameter, a high number of intrafusal fibers per spindle and numerous compound spindles, that is, clusters of spindles located closely together within a common capsule. The largest and most complex spindles are located in the deep masseter and they make the deep portion well adapted for postural mandibular control, possibly of special importance in early learning and improving speech function. In speech, precise jaw movements occur within a three-dimensional space around the mandibular postural position. Interestingly, the number of muscle spindles in the jaw muscles seems to increase in the evolutionary series from lower primates towards man (Osterlund et al 2011). Masseter muscle spindles do not undergo major changes in morphology and composition from young age to adulthood. This in turn suggests early proprioceptive demands during growth and maturation of jaw motor skills. However, the muscle spindle density was three times higher in the young than in the adult masseter (Osterlund et al 2011). An high density of muscle spindles can also be found in the temporal muscle too (Turker 2002), with a prevalence in the anterior portion of the muscle (Zhang et al 2006). This finding reinforces the idea that the jaw-closer spindles should have a strong proprioceptive impact on the control of human function. Data obtained from suprahyoid muscles confirmed the rareness of these receptors; in fact the need for MS in jaw opener muscles is reduced by their usual relationship with external forces, such as gravity. Opening of the mouth occurs in gravity favour and can be mainly controlled by the variations of jaw closer muscle tone. Additionally, jaw opener muscles, are almost never subjected to condition of stretching, because of the
limit, given to mouth closing, by the teeth contact (Saverino et al 2014). Additionally this suggest that these muscles have either alternative means of proprioceptive control. The information about the muscle length can be processed by the brain to determine the position of body parts and it plays a crucial role in regulating the contraction of muscles, by activating motor neurons via the stretch reflex to resist muscle stretch. Central connections of the periodontal mechanoreceptors are quite unique in that most of these receptors have their cell bodies in the trigeminal mesencephalic nucleus along with the spindle cell bodies. It has been suggested that, in the trigeminal mesencephalic nucleus, an electrical link may exist between the cell bodies of spindles and periodontal receptors. It is also unique that the periodontal receptors and muscle spindles from jaw muscles have direct projections to the cerebellar cortex. It is thought that this direct connection can be used as a reliable signal of tooth contact, and this may be used to zero or recalibrate the spindle afferent discharges. Muscle spindles in the jaw-closing muscles give very finely graded information regarding mandibular movement. However, they cannot give reliable information about jaw position over a long period of time, because the spindle properties and the fusimotor activity change continuously during chewing. For the normal mandibular posture to be maintained, absolute positional information is needed, and that requires calibration of the muscle spindle afferent information with the exact time of tooth contact. This calibration could be done by a comparison of the direct and reliable information received via the spindle and periodontal afferents to the cerebellum. This comparison may allow the cerebellum to alter fusimotor activity appropriately and regulate the gain of the spindles in the jaw muscles (Turker 2002).

Golgi tendon organs are composed by sensory fibers, which lose they myelinic sheaths and which are located in the tendons. These receptors sense changes in muscle tension. The Golgi tendon reflex operates as a protective feedback mechanism to control the tension of an active muscle by causing relaxation before the tendon tension becomes high enough to cause damage, while the spindle stretch reflex operates as a feedback mechanism to control muscle length by causing muscle contraction. Although the tendon reflex is less sensitive than the stretch reflex, it can override the stretch reflex when tension is great, making you drop a very heavy weight, for example. Like the stretch reflex, the tendon reflex is ipsilateral.
There is only limited evidence of the existence of tendon organs in human or animal jaw muscles. The functional connections of these afferents, if they exist, are not known (Turker 2002).

The proprioception role in the stomatognathic system is a protective role during teeth contact. In general, proprioceptive signals converge on the mesencephalic trigeminal nucleus through the mandibular branch of the trigeminal nerve. In turn, these afferent impulses are transferred to the trigeminal motor neurons to obtain precise control of jaw movements. Takeda and Saitoh (2016) made an electromyographic analysis of swallowing in order to test if swallowing could have been modified by changing the amount of proprioceptive feedback from a number of different receptors while holding a food bolus in the mouth and clenching. Initiation of the swallowing reflex was detected by an anterior shift of the thyroid cartilage using a laser displacement sensor and by submental sEMG signals. To vary the proprioceptive input, the participants were instructed to occlude their teeth at various intensities (weak, intermediate and strong) while holding the 5-ml jelly bolus on the tongue. Contractile forces of the masseter muscles during occlusion tended to correlate negatively with electromechanical delays on suprathyroid muscle contraction.

Afferents from proprioreceptors, articular receptors, mucosal and cutaneous mechanoceptors are collected by the Central Nervous System, which coordinates all the information.

c. Other receptors
Chemical receptors are situated along the whole alimentary channel and are particularly represented in the aryepiglottic fold. Gustative receptors take place in the taste buds on the tongue surface as well as on the pharyngeal area and on the palate. The pharyngeal area is sensible to the four tastes, but less than oral cavity. Taste sensory input synapses almost exclusively in the nucleus of solitary tract (NTS), but predominantly in regions rostral to the subnuclei that contain interneurons vital to eliciting swallowing. Logemann et al. (1995) measured differences between swallowing a regular barium suspension and a sour barium suspension prepared in a 50% ratio with lemon juice in patients with neurogenic dysphagia. Both oral and pharyngeal transit times were shortened with the sour bolus (Steele and Miller 2010). A sour bolus elicits more
frequent swallowing than a water or a sweet bolus (Mulheren et al 2016). Nociceptors and thermoceptors are well represented too in the stomatognatic system. Cold stimulation seems to decrease the latency to induce one swallow (Steele and Miller 2010).

1.3.2. Afferent fibers
Signals transmitted by receptors are collected by afferent fibers of cranial nerves and they are converged in the central nervous system (CNS), in order to be processed. Involved nerves are olfactory nerve (I), optic nerve (II), whose pathways are different from the nerves related to the general sensorial information. Trigeminal nerve (V) with its three branches, facial nerve (VII), glossopharyngeal nerve (IX) and vagus nerve (X), especially with its superior laryngeal branch, are involved in the sensorial path. These nerves bring their information in brain stem nuclei, trigeminal nucleus (mesencephalic, pontine and spinal tract) and solitary nucleus (SN) (VII, IX and X) (Fig 3). Trigeminal mesencephalic nucleus (next to the masticatory one) receives signals from the receptors inside the masticatory muscles and periodontal information, for masticatory regulation. The pontine (main one) one receives fine tactile information (face, lips, teeth, palate, tongue anterior part), while the spinal nucleus receives less fine tactile information as well as thermal and pain-related information. IX and X nerves bring information from the posterior region of the tongue, the pharynx and larynx to the solitary nucleus (SN) in the pontine part of the brain stem. In this context we refer to VII nerve because it brings the taste sensation of the anterior portion of the tongue, which is the transmitted to the rostral portion of the solitary nucleus (Fig 4). The superior laryngeal nerve innervates the larynx and plays a major role in initiating the swallowing reflex (Takahashi et al 2014). In fact the motor sequence can be readily initiated by the internal branch of the superior laryngeal nerve (Jean 2001). The SN plays a crucial role in the gustative sensation, but its importance in the swallowing task has been demonstrated as well. Stimulation applied to the solitary tract and its nucleus can induce a very similar swallowing pattern to that obtained in response to the superior laryngeal nerve stimulation (Jean 2001).
1.3.3. Central pattern generator (CPG)

Once the afferent fibers reach the nuclei in the brainstem, the information is led to specific areas which are known as swallowing center and they represent the first central swallowing integration area. Swallowing is a complex but stereotyped motor sequence with a fixed behavioral pattern. It constitutes, however, one of the most elaborated
motor functions, even in humans, since it requires the coordination of more than 25 pairs of muscles in the mouth, pharynx, larynx and esophagus. The sequential and rhythmic patterns of swallowing are formed and organized by a central pattern generator (CPG). The CPG can be subdivided into three systems: an afferent system corresponding to the central and peripheral inputs to the center; an efferent system corresponding to the outputs from the center, consisting of the various motoneuron pools involved in swallowing and an organizing system corresponding to the interneuronal network that programs the motor pattern.

a. Motoneurons

Motoneurons are localized within the trigeminal (V), facial (VII), and hypoglossal (XII) motor nuclei, the nucleus ambiguus (IX, X), the dorsal motor nucleus of the vagus (X) and at the cervical spinal level between C1 and C3. These motor nuclei do not all participate to an equal extent in swallowing, at least during the basic pattern. V and VII motor nuclei do not deal mainly with swallowing. They are most strongly involved in several other orofacial activities such as jaw reflexes, mastication, licking, and sucking. Lesion experiments have shown for example that abolishing the V motor nuclei does not affect the swallowing sequence. Trigeminal motoneurons mainly involved in swallowing innervate the mylohyoid, anterior digastric, lateral pterygoid, and tensor veli palatini. Within the VII motor nucleus, motoneurons greatly involved in swallowing control the posterior digastric and stylohyoid. Other muscles innervated by these two motor nuclei, such as the medial pterygoid, temporal, and masseter are more facultative swallowing muscles. In fact, the main motor nuclei involved in swallowing are the XII motor nucleus and the nucleus ambiguous (Jean 2001).

b. Interneurons

The swallowing neurons are located in two main brain stem areas: 1) in the dorsal medulla within the nucleus tractus solitarii (NTS) and in the adjacent reticular formation, where they form the dorsal swallowing group (DSG) and 2) in the ventrolateral medulla, just above the nucleus ambiguus, where they form the ventral swallowing group (VSG).
A) DSG. Within the NTS, there exist neurons that fire during either the oropharyngeal or the esophageal phase of swallowing. Most of the oropharyngeal neurons are active either just a few milliseconds before or during the oropharyngeal phase of swallowing (Jean 2001).

B) VSG. In the ventrolateral medulla above the nucleus ambiguus, there also exists a large population of oropharyngeal swallowing neurons. These neurons have been identified as interneurons. The burst firing behavior of the VSG neurons is very similar to that of the DSG neurons in terms of the sequential firing pattern; this population has, however, a lower instantaneous discharge frequency.

Additionally, within V and XII motor nuclei or in their close vicinity a groups of interneurons has been identified as premotor neurons or neurons involved in the bilateral coordination of the motoneuronal pools (Jean 2001).

1.3.4. Neural network

Within the swallowing network (Fig 5), VSG neurons are activated via DSG neurons and motoneurons are driven by neurons of the VSG. If we therefore consider the swallowing CPG, there exist simple circuits linking together the afferent fibers, the DSG neurons, the VSG neurons and the motoneurons. It has been established in several networks involved in basic motor behavior, such as locomotion, that within a given CPG all the neurons are not equal since some of them play a preeminent role. As regards swallowing, data already obtained suggest that neurons in the DSG are likely candidates to act as generator neurons in the initiation and organization of the sequential or rhythmic motor pattern. The VSG contains the switching neurons, which distribute the swallowing drive to the various pools of the motoneurons involved in swallowing. The swallowing network in mammals therefore provides a unique example of neurons located within a primary sensory relay, i.e., the NTS, which nevertheless play the role of generator neurons. Several lines of evidence support the idea that NTS neurons play a leading role in swallowing. NTS neurons exhibit a sequential or rhythmic firing pattern that parallels the motor pattern. As this firing remains unaltered after complete motor paralysis, it is clear that it is actually a centrally generated premotor activity. Moreover, most of the neurons, if not all those which have a preswallowing activity, are located
within the NTS (Jean 2001). In fact right before swallowing an activation of premotor neurons in the intermediate (NTSim), ventromedial (NTSvm) and interstitial (NTSis) subnuclei of the NTS can be seen. The primary NTS premotor subnuclei that control the pharyngeal phase of swallowing are the NTSis and NTSim (Lang 2009). In addition, systematic exploration of the brain stem with concentric bipolar electrodes to determine which central structures responded to stimulation by triggering swallowing have shown that the active points are situated only in the region of the solitary complex. It may be stated that swallowing results from the stimulation of afferent fibers belonging to the solitary tract.

The CPG for swallowing consists of two hemi-CPGs, each located on one side of the medulla. The existence of two hemi-CPGs was established by making longitudinal midline sections of the medulla. After this splitting, stimulation applied to the SLN on one side triggered a “unilateral swallowing,” i.e., a swallowing sequence involving only the ipsilateral oropharyngeal muscles, except for the middle and inferior pharyngeal constrictors in some species. These results indicate that under physiological conditions, the two hemi-CPGs are tightly synchronized and organize the coordinated contraction of the bilateral muscles of the oropharyngeal region (Jean 2001). The swallowing motor sequence is mainly generated in the ipsilateral hemi-CPG and this CPG transfers the swallowing pre-motoneuron signals to the contralateral CPG (Jean 2001; Ertekin and Aydogdu 2003) (Fig 6).
Figure 5: diagram of the swallowing central pattern generator (CPG). The CPG includes two main groups of neurons located within the medulla oblongata: a dorsal swallowing group (DSG) located within the nucleus tractus solitarii (NTS) and the adjacent reticular formation and a ventral swallowing group (VSG) located in the ventrolateral medulla (VLM) adjacent to the nucleus ambiguous. The DSG contains the generator neurons involved in triggering, shaping and timing the sequential or rhythmic swallowing pattern. The VSG contains the switching neurons, which distribute the swallowing drive to the various pools of motoneurons involved in swallowing (Jean 2001).

Figure 6: schematic representation of the central pattern generator of swallowing. Peripheral and supramedullary inputs reach to and around nucleus tractus solitarius–dorsal swallowing group (NTS-DSG). NTS-DSG activates the ventral swallowing group of premotor neurons in the ventrolateral medulla–ventral swallowing group (VLM-VSG) adjacent to the nucleus ambiguus (NA). VLM-VSG drives the motoneuron pools of the V, VII, IX, X, XII, C1–3 CN bilaterally (Ertekin and Aydogdu 2003).

1.3.5. Superior swallowing control

Experimental data have shown that the basic swallowing pattern can be induced without any supramedullary structures being involved. Under physiological conditions, however, the swallowing network receives inputs from higher centers and several cortical and subcortical structures can also influence the pattern of swallowing. In addition, the descending pathways can initiate or modify the pattern of swallowing.
through interaction with the peripheral inputs to the brain stem. Thus, although not generally susceptible to central control, the patterned sequence of events associated with swallowing can be modified by learning, for example following surgery for carcinoma of the larynx (Lavelle 1988). It is possible that during repeated swallowing, descending signals from the cortical sites associated with swallowing decrease the threshold to evoke swallowing (Ertekin and Aydogdu 2003). The fact that an individual can swallow voluntarily without the existence of any need to ingest food or to protect the upper airways shows that the medullary swallowing network can be activated at least by inputs from the cerebral cortex. In addition, several clinical reports have indicated that cortical dysfunction may result in dysphagia or swallowing impairments or may affect esophageal peristalsis (Jean 2001). The triggering of the spontaneous swallows probably does not require cortical drive but could involve communication with the cortex and subcortical regions, and can occur between meals and during non-REM sleep and depends on the amount of saliva accumulated in the mouth (Ertekin and Aydogdu 2003). These observations point to the involvement of supramedullary influences, although the peripheral afferent pathway and the CPG seem to remain unaltered in these patients. Supramedullary structures may be responsible for various effects on swallowing such as initiating the motor activity, or modulating reflex swallowing. There exist a number of subcortical sites, including the corticofugal swallowing pathway, which can trigger or modify swallowing, in particular the internal capsule, subthalamus, amygdala, hypothalamus, substantia nigra, mesencephalic reticular formation, and monoaminergic brain stem nuclei. These influences can be either excitatory or inhibitory. It has been reported that several forebrain regions, including the amygdala and the lateral hypothalamus, may facilitate swallowing by means of dopaminergic mechanisms. Inhibitory effects can be evoked by stimulating brain stem structures such as the periaqueductal gray, the ventrolateral pontine reticular formation, and some monoaminergic cell groups. Results suggest that these inhibitory effects probably involve opiate and monoaminergic mechanisms at the level of the NTS. Whether these influences act directly on the medullary CPG or may involve a more complex central pathway is not known. Few studies have in fact dealt with these central effects on the neurons of the CPG. As far as the supramedullary influences on swallowing and their action at the cellular brain stem level are concerned, all the results available so far have
been obtained in studies on the cortical influences on swallowing. However lesion of the NTS area involved abolishes the swallowing evoked by cortical stimulation, which further indicates that the solitary system is the main central system responsible for swallowing. Results obtained on anesthetized sheep indicate that most of the early neurons in the DSG can be activated by applying cortical stimulation. This is in agreement with the idea that one of the functions of the cortical area may be to trigger the “voluntary” swallowing motor sequence. Cortical stimulation induces an initial activity followed by the swallowing burst that accompanies the onset of swallowing. Early neurons in the DSG were cortically activated with a shorter latency than those in the VSG and only 32% of all the neurons in the ventrolateral medulla were cortically activated. Late neurons in the DSG also responded to cortical stimulation, but in small numbers (38%) and with a longer latency. None of the late neurons in the VSG nor the very late neurons either in the DSG or the VSG was activated by cortical stimulation. On the basis of the finding that during swallowing sensory feedback is conveyed to the cortical area via a first relay in the pons, the swallowing cortical area may have a further function. Neurons in this area may belong to a ponto-cortico-medullary loop so that upon receiving sensory information, they might control the activity of the CPG swallowing neurons as they fire successively, just as peripheral afferent fibers do. It has been shown that cortical neurons in the swallowing cortical area of sheep are activated or inhibited during swallowing. Therefore, the cortical swallowing area may serve mainly to trigger swallowing and control the beginning of the motor sequence, after which the sequence might be carried out without any further cortical control (Jean 2001). The swallowing tasks yield activation of the lateral postcentral gyrus localized to Brodmann’s area 3, 2, 1 and/or 43. This finding of swallow-related activation of the postcentral gyrus might reflect various types of oropharyngeal sensory processing and underscore the importance of afferent information in the regulation of swallowing. Cortical activation during both swallowing and swallowing-related motor tasks that can be performed independent of swallowing was also found in the parietooccipital region corresponding to Brodmann’s areas 7, 9 and 31. Somato-sensory and parietal regions have been cited as a region of activity during mechanical and chemical stimulation of the esophagus The somato-sensory cortex and posterior parietal cortex are likely to have a sensory role in the control of swallowing. Also the temporal lobe has been implicated
in a number of functions that are related to swallowing (Ertek and Aydogdu 2003). Speaking about efferent signals, human data show that the locus of the cortical control of swallowing lies within and antero-caudal to the face area of primary motor cortex. A number of brain regions with increased activation were detected with PET (Hamdy et al 2000). The cerebral motor cortex is the portion of the cortex which is responsible for the planning, the regulation and the execution of the voluntary movements. The precentral area is situated in the precentral gyrus and includes the anterior walls of the central sulcus and the posterior part of the frontal gyri. The precentral area may be divided into posterior and anterior regions, the first one, which is referred to as the motor area, primary motor area, or Brodmann area 4, occupied the precentral gyrus. The anterior region is known as the premotor area, secondary motor area, or Brodmann area 6 and parts of areas 8,44 and 45 (Fig 7).

The primary motor area receives numerous afferent fibers from the premotor area, the sensory cortex, the thalamus, the cerebellum and the basal ganglia. The primary motor cortex is not responsible for the design of the pattern of movement but it is the final station for conversion of the design into execution of the movement. The premotor area receives inputs from the sensory cortex, the thalamus and the basal ganglia; its function is to store programs of motor activity assembled as the result of past experience. It can be reported that the premotor area programs the activity of the primary motor area. In
terms of human swallowing, there might be two distinct patterns of activity: first, the caudolateral motor cortex, which may be associated with the initiation of the full swallowing sequence at the highest level and second, the premotor regions which may be more modulatory and concerned with “priming” the pharyngoesophageal components of swallowing. The supplementary motor area represented in the superior and middle frontal gyri, is believed to be associated with motor planning and, in particular, with planning of sequential movements as occurs with oropharyngeal swallowing. In addition the activation of the anterior cingulate cortex during volitional swallowing may reflect the attentional and/or affective component of the swallowing task. Finally studies have demonstrated how the insula and the frontal operculum are activated during swallowing (Ertekin and Aydogdu 2003).

Swallowing recruits multiple cerebral regions, often in an asymmetrical manner, particularly in the insula, which is predominantly on the right, and in the cerebellum, being mainly on the left. These latter observations are in keeping with the earlier observations that motor cortex representation for swallowing musculature displays degrees of asymmetry (Hamdy et al 2000). Studies conducted with the technique of transcranial magnetic stimulation showed that in the majority of the individuals, the projection from one hemisphere tended to be larger than that from the other, i.e. there was an asymmetric representation for swallowing between the two hemispheres, independent of handedness (Hamdy et al 1996, 2000). The new technological advances in functional imaging of the human brain have revolutionized our understanding of how the cerebral cortex operates in processing sensory and motor information. In particular, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have become established as useful methods for exploring the spatial localization of changes in neuronal activity during tasks, within both cortical and subcortical structures.

1.3.6. Neural mechanism

Speaking about a swallowing center is quite an oversimplification. A voluntary swallow (VS) can be distinguished from the spontaneous one (SS). In VS, the regions of the cortex and subcortical areas involved with swallowing serve mainly to trigger swallowing and to control the beginning of the motor sequences (i.e., mainly the oral
phase of swallowing). After this, sequential muscle activation is carried out without any further cortical control to perform the pharyngeal and esophageal phases. VS occurs with a desire to eat or drink such as during mealtime and while awake and aware. Although saliva swallowing alone cannot precisely distinguish the kind of swallowing, SS is mostly a kind of saliva swallow that occurs without the person being aware, such as between meals and during sleep. The major difference between the VS and SS is the origin of the swallow trigger. Materials in the mouth (food or saliva) and the cortical drive to the tongue and the submental muscles are necessary for initiation of VS. On the other hand, according to the classical view of the initiation of SS, the oral phase is bypassed. Despite this, it has been shown in animals that the perioral, submento and lingual striated muscles can also be activated in SS, probably under the control of the medullary network of the CPG and bypassing the cortical drive. Direct human physiological studies are necessary to investigate those muscles during SS. Although the initiation of the oropharyngeal phase of swallowing is different between VS and SS, the subsequent sequential and stereotyped motor activity of the pharyngeal and esophageal phases are under the control of the brainstem network for both types of swallows. VS and SS can be differentiated in terms of state of awareness, wakefulness and to what extent the oral phase is involved. In VS, there is clearly a reflexive pharyngeal phase, as in SS. However, there is no strong evidence of a cortical influence on SS in human (Ertekin 2011).

It is likely that modulation of the process of swallowing results from activity in cranial nerves other than those directly related to swallowing. For instance, salivatory preparation of the bolus cannot occur in the absence of cholinergic activity mediated through the peripheral and autonomic nervous system. Also, the striated muscles mediating swallowing in the pharynx and upper one-third of the esophagus, are under the control of impulses originating in motor neurons of the corresponding cranial nerve nuclei, whereas the smooth musculature associated with swallowing are innervated by cholinergic vagal preganglionic fibers that synapses with a plexus in the muscle itself, resulting in postganglionic release of acetylcholine (Lavelle 1988). The swallowing center is also said to be linked to the respiratory center, for respiration is transiently suspended in swallowing. Such linkages are certainly likely because all these centers lie very close together, but the exact pathways are not known (Jean 2001).
Sensory peripheral information are led to the brainstem as well as to the sensory cortex of the brain. In response to a peripheral stimulus, or central command to swallow, the neuronal program of the swallowing center exerts a sequential all-or-none pattern of excitatory and inhibitory effects of the various motoneurons supplying the muscles of swallowing (Fig 8-9). It is believed that sensory feedback originating from the oropharyngeal mucosae and deeper receptors in the region may modify the CPG of the bulbar swallowing network. However, there has been much debate about the effects of mucosal receptors on oropharyngeal swallowing, because of the discrepancy among the studies of topical anesthesia of the oropharynx. On the other hand, the sensory deficit in the oropharyngeal mucosae has been proven to be one of the important causes of dysphagia and aspiration in stroke patients. Results obtained during topical anesthesia of the oropharyngeal mucosae in human subjects suggest that adequate sensory inputs are necessary for the perception of the bolus volume and viscosity by the cerebral cortex and the bulbar swallowing network. The insufficiency of the sensory coding would produce an “uncertain evaluation” in the central nervous system. The main role of the oropharyngeal mucosal receptors may be to contribute to the initiation of swallowing, but when swallowing is triggered, the pattern and sequential activity of swallowing is not essentially changed (Ertekin and Aydogdu 2003). The efferent portion of the CPG coordinates impulse flow for the Trigeminal, Glossopharyngeal, Vagal and Hypoglossal nerves for soft palate contraction, for pharyngeal constriction, for laryngeal muscles contraction, for tongue muscles contraction and for esophageal muscles contraction. In detail, when the movement of the bolus from the oral cavity to the pharyngeal spaces triggers the swallowing reflex or response, the following physiological events occur in rapid overlapping sequence. All of the events until the esophageal phase are mainly controlled by the CPG of the brain stem. The nasal, laryngeal and tracheal airway is protected by several “reflex” events including closure of the velopharyngeal isthmus by the palate, laryngeal elevation and suspension by suprahyoid/submental muscles and closure of the larynx by laryngeal muscles of the vocal folds and epiglottis. Protection of the airway is essentially owing to the CPG.
Figure 8: sensory pathways and swallow response integration. Schematic representation of the neuronal pathways perceiving stimuli in the oropharynx and larynx that lead to the integration of all the information as the swallow response in the central pattern generator in the brain stem (Alvarez-Berdugo 2016).

Figure 9: schematic of the anatomic physiology in normal swallowing. There are three different locations in the swallowing function: The first location is the peripheral machine of swallowing process known as the oropharynx where all of the peripheral sensory/motor events occur. The second location is the medullary swallowing center in and around the reticular network of nucleus tractus solitarius (NTS) and nucleus ambiguus (NA) at the ponto-bulbar region known as the Central Pattern Generator (CPG). The CPG receives all peripheral inputs and descending motor drives. The third location is the cerebral cortex and some subcortical structures. These are connected to the brainstem CPG especially via corticobulbar pathways (Ertekin 2011).

The CPG must be able to perform a wide range of tasks, such as converting the repetitive messages conveyed by central or peripheral afferent inputs into a bursting activity, transmitting bursting activity after a variably long delay depending on the nature (oropharyngeal or esophageal) of the neuron in the network, ensuring via a rostrocaudal inhibitory mechanism that the later neurons cannot fire before or during the
activity of the earlier neurons, and generating a rhythmic bursting pattern, at least in the case of the oropharyngeal neurons. The bursting pattern of the swallowing neurons may be built up as the result of either re-excitation phenomena or excitatory feedback mechanisms. The centrally generated bursting pattern of swallowing NTS neurons suggests that these mechanisms may well take place within the DSG. Neurons with pre-swallowing activity presumably possess these re-excitation loops so that the neuronal firing will increase until reaching the critical level at which the swallowing burst is generated. In addition to the mechanisms by which is generated the burst firing of the swallowing neurons, the swallowing network can be viewed as a linear-like chain of neurons based on the rostrocaudal anatomy of the swallowing tract (Fig 10). Because there exist, within the NTS, neurons which fire sequentially during swallowing, each neuron or group of neurons in this chain may control more and more distal regions of the swallowing canal and be responsible for the successive firing behavior. Excitatory connections between neurons may provide the basis for the successive excitation of the cells via increasingly numerous polysynaptic connections. In addition to the central connections, each DSG neuron in the chain may be synaptically activated via peripheral afferent fibers originating in the corresponding part of the tract which is under their control. Whether or not this linear pattern of organization in the form of a chain of neurons exists at the VSG level also has not yet been ascertained. In addition to excitatory connections, there also exist inhibitory connections between the various links in the chain. It has been reported that when the neurons responsible for the beginning of the swallowing sequence fire, the cells controlling the more distal parts of the tract are inhibited, and their activity is delayed. Inhibitory phenomena play an important role in the shaping and timing of the sequence, since the neurons controlling more distal parts of the swallowing canal are subjected to longer periods of inhibition than those controlling rostral parts. Inhibitory mechanisms may not only be responsible for delaying the onset of neuronal firing, but they may also contribute directly to the sequential excitation of the neurons. In fact, via mechanisms such as disinhibition or post-inhibitory rebounds, the inhibitory connections may be at least partly responsible for the progression of the contraction wave. With the assumption that there exist only inhibitory connections between swallowing neurons the sequential excitation of neurons
may result from post-inhibitory rebounds involving in this case cellular properties of the neurons in addition to network connections.

Figure 10: possible mechanisms involved in pattern generation. A: the swallowing network can be viewed as a chain of neurons (1-4) that parallels the rostrocaudal anatomy of the swallowing tract. When triggered by peripheral or central inputs, the neurons at the beginning of the chain (1) generate, on the basis of network and/or cellular properties, a burst firing which starts the sequence; they also exert inhibitory effects on the more caudal neurons in the chain (2, 3, and 4) through central inhibitory connections (lines with black dots). The swallowing drive may be transferred by central excitatory connections (white triangles) to the subsequent neuron in the chain (2), which also inhibits the more caudal neurons, etc. The sequential firing behavior therefore results from the successive excitation of the neurons paralleled by a rostrocaudal inhibition, which lasts longer when the neuron is more distal in the chain.

This network may function without any sensory feedback, but the peripheral inputs may modulate the sequence (broken lines). Intrinsic properties of the neurons such as pacemaker, post-inhibitory rebound, and delayed excitation properties may intervene in the functioning of the chain. Given this scheme of organization, it can be postulated to account for the differences between primary and secondary peristalsis, that the strength of the central excitatory connections decreases in the caudal direction, while the afferent feedback inputs then become more important. B: the chain may also function mainly on the basis of inhibitory connections. In this case, the sequential firing of the neurons may be produced mainly by mechanisms such as post-inhibitory rebounds. In this case again, these mechanisms will presumably decrease as they occur more caudally. As suggested by field potential recordings, it can be postulated that the neurons generating the oropharyngeal stage can also induce, in addition to the inhibition, a long-lasting excitatory influence on neurons of the esophageal network, indicated here by the dotted line with striped triangles (see text for further information). The sequential firing is therefore dependent on both the inhibitory connections and the properties of the neurons and on a facilitatory influence exerted on the esophageal network in the case of the primary peristalsis. This tentative mechanism of intrinsic modulation may account for the differences between primary and secondary peristalsis. The traces below the diagrams give the membrane potential of oropharyngeal (1) and esophageal neurons (2, 3, and 4). The upward deflection corresponds to a depolarization of the neuron and the downward deflection to an hyperpolarization. The dotted lines in B show a possible long-lasting excitatory or facilitatory influence exerted by the oropharyngeal neurons on the esophageal ones (Jean 2001).

However, there exist some differences between the oropharyngeal and esophageal phases of swallowing. In fact, results indicate that unlike the oropharyngeal phase, the esophageal phase may show some lability and also suggest that the central program
controlling this phase may be less robust than that responsible for the oropharyngeal phase. The size of the neuronal population involved during these two phases is also different. Depending on the number of muscles involved, the number of active neurons during the oropharyngeal phase of swallowing has been found to be far larger than that involved in the esophageal phase. The bursting activity of the oropharyngeal neurons is also very different from that of the esophageal neurons. Differences may reflect differences in the strength of the synaptic connections along the chain of neurons, possibly due to the existence of increasing numbers of synapses, and/or to the properties of the cells. In fact, data obtained in the case of secondary peristalsis have suggested that the swallowing CPG can be subdivided into two subnetworks: an oropharyngeal and an esophageal net of neurons, each of which mediates the patterning of the respective phase of swallowing, but that the esophageal net is likely to have less robust central mechanisms and be more dependent on afferent inputs. The swallowing CPG is not an automatic, continuously functioning CPG and the question arises as to whether the swallowing neurons are completely inactive when no swallowing occurs, or whether these neurons may have other functions. This question is especially interesting, since we have established that swallowing neurons include NTS neurons and it has by now emerged that the NTS is far from being simply a sensory relay. NTS neurons are involved in many activities, such as the autonomic ones, as well as in endocrine processes and in several integrated behaviors such as emotional processes, hunger, thirst, control of pain mechanisms, regulation of the level of consciousness, and probably many other yet non identified functions. Are all the NTS neurons in this very small population each devoted to a single fixed function, or do there exist within the NTS one or several populations of neurons that are flexible and participate in several functions, depending on the inputs they receive? When a given stimulus is delivered, a pool of appropriate neurons is activated and forms the swallowing CPG, whereas these neurons are involved in other tasks when no swallowing activity is required. It has been established that not only motoneurons, but also interneurons can be involved in at least two different tasks, such as swallowing and respiration, swallowing and mastication, or swallowing and vocalization. Motoneurons presumably receive a drive from separate CPGs, and their activity no doubt depends on synaptic interactions between the two networks. Some recent results have indicated that interneurons localized in the dorsal
(DSG) or ventral (VSG) regions of the swallowing network also fire during several motor behaviors such as swallowing, respiration, mastication and vocalization (Jean 2001).

1.3.7. **Muscular coordination**

The importance of swallowing in transport and protection is illustrated by the frequencies: 2400 swallows per day increasing to 300/h during eating (Lavelle 1988). Such activity is aided by contraction and coordination of the muscles of different portions of the body. In mammals, all the muscles involved in the oropharyngeal stage are striated and are therefore driven by several pools of motoneurons located in various cranial motor nuclei in the brain stem and the uppermost levels of the cervical spinal cord. The esophageal muscles are composed of both striated fibers and smooth ones, which are controlled by the autonomic nervous system. The oropharyngeal stage of the basic or fundamental swallowing is a complex, stereotyped sequence of excitatory and inhibitory events. It involves a set of muscles that always participate in this fundamental motor pattern and therefore, these muscles have been termed obligate muscles. In addition to the obligate muscles, other muscles, such as extrinsic tongue muscles, facial muscles, lip muscles and levator mandible muscles, may or may not participate in swallowing, depending on either the species or the swallowing conditions, and therefore constitute facultative swallowing muscles. Muscular events are described below (Jean 2001):

- The oral phase of swallowing recruits the jaw closing muscles of the mandible (i.e. temporalis, masseter and medial pterygoid) to stabilize the mandible. As a result, the type of bolus affects the recruitment of the jaw closing muscles (Ertekin and Aydogdu 2003).

- The perioral-facial muscles are the first recruited during the oral phase of swallowing to provide an anterior seal of the lips. In healthy human subjects, orbicularis oris and buccinator muscles firmly close the mouth to prevent food from escaping, flatten the cheeks and hold the food in contact with the teeth. It has been observed that the perioral muscle activity is ended just before the pharyngeal phase of swallowing, while the masseter activity can continue or reappear during the pharyngeal phase of swallowing (Ertekin and Aydogdu 2003) (Fig 11).
The styloglossus and hyoglossus muscles force the root of the tongue against the soft palate and posterior pharyngeal wall (Lavelle 1988).

The tongue movements that initiate swallowing require the concomitant contraction of the mylohyoid, geniohyoid and digastric muscles (submental muscles- SM) (Lavelle 1988) (Fig 12).

SM contraction initiates swallowing and it continues until the completion of the oropharyngeal swallowing process, since they pull up the hyoid bone into an anterosuperior position, which elevates the larynx and initiates other reflexive changes that constitute the pharyngeal phase. As we will largely describe, when a swallow is initiated voluntarily, the contraction of the SM muscles should be controlled by at least two routes. During the initial part, SM muscles should be activated by the cortical drive either directly or via the brain stem CPG. The latter part of SM muscle activation should, however, be controlled by the CPG of the brain stem network, especially in the period immediately after the onset of laryngeal upward movement, which is an important and early event of the pharyngeal phase in voluntarily induced swallowing. In many of the dysphagic patients, the onset of SM is extremely prolonged, which indicates the difficulties of the cortically induced triggering mechanism due to the involvement of the corticobulbar fibers (Ertekin and Aydogdu 2003). SM activity seems to be influenced by the body posture in upright position the electromyographical activity was larger than the one detected in supine position (Shiino et al 2016).

The elevator and tensor veli palatine muscles elevate the soft palate, with additional shortening and dorsal thickening until approximation against the posterior pharyngeal muscle.

The middle and inferior pharyngeal constrictor muscles narrow the hypopharynx and contributes to the peristaltic wave.

The epiglottis tilts dorsally and downward, thanks to muscular elevation of the larynx and the contraction of the floor of the mouth (submental muscles), along with elevation and posterior movement of the hyoid bone (Lavelle 1988).

CP muscle is a striated muscle sphincter situated at the pharyngoesophageal junction. It is one of the most important muscles for the evaluation of neurogenic dysphagia The CP sphincter muscle is organized with motoneurons
both tonically and phasically activated. The muscle is tonically active during rest and this continuous activity ceases during a swallow in human subjects. During a swallow, tonic motoneurons supplying the CP muscle are first inhibited and the CP sphincter is relaxed. Consequently, during the rebound burst, phasic larger motoneurons fire transiently to close the sphincter as fast as possible after passage of the bolus and the tonic motoneurons are re-excited. Although both units are under the control of the CPG, both are also influenced by sensory and cortical inputs (Ertekin and Aydogdu 2003) (Fig 13).

Figure 11: Representation of the jaw closing muscles (A-C) and some of the perioral muscles (D-E): A: masseter muscle, B: temporalis muscle, C: medial pterygoid muscle, D: orbicularis oris muscle, E: buccinators muscle (from Gray’s anatomy, 2009).
Figure 12: Representation of the submental muscles (A-C) and the tongue muscles (D-E): A: digastric muscle, B: genyohyoid muscle, C: mylohyoid muscle, D-E: tongue muscles from different views (from Gray’s anatomy, 2009).

Figure 13: representation of the pharyngeal muscles (A) and the larynx structure (B and C) (from Gray’s anatomy, 2009).
1.4. Swallowing process evaluation

The analysis of the swallowing process is an important phase during the clinical evaluation of the stomatognatic system. It is stated that this physiological task can occur more than 2400 times per day and thus it is important to evaluate its progress step by step. There are many clinical conditions related to a non-physiological swallowing mechanism, such as atypical swallowing or dysphagia. The most important phase of the clinical pathway is the right diagnosis, to ensure the morphology of the different structures involved, the muscular coordination and the neural integrity. A complete examination as well as an anamnestic questionnaire are crucial stages in the diagnostic process.

In addition also instrumental analysis can be used. Many methods can be listed; among all the techniques, the videofluoroscopy (VF), the fiberoptic endoscopic evaluation (FEE) and the surface electromyography (sEMG) are the most used (Yu et al 2013). The videofluoroscopy is a radiologic exam, in which the passage of the bolus from the oral cavity to the stomach is evaluated. The patient is asked to eat a contrast medium and thus, after x-rays exposition, the passage of the bolus is monitored thanks to a connected monitor (Fig 14).

![Figure 14: an example of videofluoroscopy during swallowing.](image)

The endoscopy was introduced since 1988 as an alternative to the videofluoroscopy. It is composed by a flexible endoscope, which passes transnasally to obtain a superior view of the pharynx and larynx. Although FEE does not show swallowing directly, the presence of subglottic residue and oropharyngeal secretion indirectly indicates the wrong progression of the movements (Umay et al 2013) (Fig 15).
Furthermore, sEMG is another method that provides important information about the muscles involved during swallowing. sEMG is a non-invasive, radiation free and cheap diagnostic technique, which gives information about muscular activity. It registers the myoelectric signal, derived from the electric field generated from muscle fibers depolarization and transmitted to skin thanks to surface electrodes attached to skin. For this reason it is suitable only for superficial muscles. It was demonstrated how electromyographic potentials in the muscle groups that are effective in swallowing can work reliably (Vaiman et al 2004; Umay et al 2013) (Fig 16).

The main advantages of VF are the integrated observation of all the swallowing phases, that is, observation of the oral preparatory phase and oral transit phase, of the elevation and anterior displacement of the hyoid-larynx complex, of the upper esophageal sphincter opening and of esophageal transit (Da Silva et al 2010). Nevertheless VF gives qualitative analysis about the swallowing process and the evaluation of the results is subjective, in addition there are some disadvantages such as the necessity of patient cooperation and transport, failure to directly show the nasopharyngeal, oropharyngeal and laryngeal anatomy, evaluation of only motor ability, risk of aspirating the opaque
material and risk of exposure to serious radiation in repeated applications. FEE is reported to be safer, more sensitive and better tolerated than VF. Moreover, the diagnosis and treatment of dysphagia can be ensured without the cooperation and mobilization of the patient (Umay et al 2013). Despite a lack of a general consensus, FEE seems to be as an alternative to VF. There have been many controversial opinions on the clinical application of sEMG. The major concern was related to the lack of reliable criteria for comparative and longitudinal evaluations.

In fact electromyographical signal is influenced by many factors: electrodes type and position, head and back position, muscular cross-talk etc (Suvinen et al 2009). Nevertheless thanks to standardized protocols, the cited factors are limited and so quantitative comparisons, both comparative (between muscles, between subjects) and longitudinal (Suvinen et al 2009; Sforza et al 2011). Considering the muscles related to the stomatognatic system standardized protocols have been developed, for masseter, temporalis, sternocleidomastoid muscles (Ferrario et al 2000; Botelho et al 2011; Frongia et al 2013). Studies for submental muscles assessment are needed.
2. RESEARCH PROTOCOL

2.1. Introduction and rationale of the study

As it is largely explained swallowing is a physiological task, which is crucial for everyday life and which is repeated thousands of time per day. It requires a complicated neuromuscular coordination, of both reflex and automatic activities, occurring during the pharyngeal and esophageal phases. A disequilibrium in the neural circuits as well as uncoordination of the active muscles are linked to many clinical situations resulting in dysphagia. During everyday life the automatic sequence of swallowing is anticipated by a first phase, which is partially under voluntary control, known as the oral phase. It mainly involves jaw closing and opening muscles, cheek and extrinsic tongue muscles. From a muscular point of view an instrumental, easy and non-invasive assessment of the physiologic pattern of activated muscles is needed. Once the protocol will be developed and the muscular model will be found, it will be possible to apply it to different clinical situations, aiming to find deviations from the physiological movement and to optimize the multidisciplinary treatment through a personalized measure of the function. As it is reported the surface electromyographic potentials in the muscle groups that are effective in swallowing can work reliably. Surface electromyography (sEMG) can detect the activation of superficial muscles and thus considering swallowing it is ideal for the analysis of the oral phase. Among jaw closing muscles, the masseter and the temporalis muscles activity has been largely electromyographically studied in a static task (maximal voluntary clenching MVC) and standardized protocols are well assessed (Ferrario et al 2000); on the contrary standardized and repeatable protocols for the submental muscles (jaw opening muscles) activity are still needed. Different studies have electromyographically analyzed the submental muscles, but without a proper standardization test, for example considering the effortful swallow as the maximal contraction for submental muscles (Reyes et al 2014). In addition the analysis of dynamic oral functions, such as swallowing, is still an open field of research and further scientific approval is needed before clinical applications.

The purposes of the current study were therefore firstly to develop a standardized protocol for the assessment of the SM during a simple static task, like MVC (phase 1). Secondly we evaluated the contribution of masticatory muscles during the oral phase of
swallowing (phase 2). Since a method is still lacking we decided to analyze a “controlled” kind of swallowing, asking the subjects to keep their teeth in contact during the function. Thus a repeatability of the mandible position and a muscular recruitment model may be drawn, even though the requested task is a not fully physiological one. Once the protocol will be developed, it will be applied to a “more physiological” kind of swallowing, asking the subjects to swallow in their most comfortable way, without giving them instructions about teeth position. Thus speculations about the activation and the symmetry of masticatory muscles during the oral phase of swallowing in healthy subjects can be done. In the future the method could be applied on different clinical situations (atypical swallowing, dysphagia, patients undergoing prosthetic rehabilitation) in order to compare their muscular pattern, to find possible alterations aiming at the optimization of the treatment, by a personalized measure of the function.

2.2. Material and methods

2.2.1. Study design

Standardized cutaneous myoelectric activity of Anterior Temporalis, Masseter and Submental muscles was recorded.

- Phase 1: preliminary measurements for the development of a standardized sEMG protocol for the assessment of submental area muscles during maximal voluntary clench (MVC).
- Phase 2: electromyographic analysis of the oral phase of swallowing and preliminary detection of the normality values in terms of duration of activation of each registered muscle, duration of the whole exercise and intensity of activation of each muscle. In addition a graphical analysis of the EMG signal will be conducted in order to assess the intensity and the position of the spike of activation of each muscle. The analyzed parameters will lead to the assessment of the reproducibility and variability of the used protocol and thus the sample size for the detection of normality values in the population will be calculated.
2.2.2. Subjects (for phase 1 and 2)

Twenty young adult subjects (10 males, 10 females, age range 19–35 years, mean 25, SD 5), volunteered for the study after a detailed explanation of the experimental protocol and possible risks involved. The study protocol was approved by the local ethic committee. During the muscles function recording, the environment was tranquil, quiet and with low light. The subjects sat in a comfortable office type chair, having a straight posture, with the feet flat on the floor, and arms resting on their legs.

Inclusion criteria (for phase 1 and 2)

- Systemically healthy subjects
- No diseases of the stomatognatic/neck areas
- At least 28 permanent teeth
- Bilaterally I Angle class (both canine and molar)
- overjet and overbite between 2- 5 mm
- no anterior or lateral crossbite
- no periodontal disease
- no recent craniofacial trauma
- no temporomandibular disorders
- no neuromuscular diseases

Exclusion criteria (for phase 1 and 2)

- simultaneous orthodontic therapies
- oral surgery within 3 months
- drugs which could interfere with the musculo-skeletal system

2.2.3. Instrumentation

Electrode type and positioning

The Anterior Temporalis (TA), Masseter (MM) and Submental area Muscles (SM) of both sides (left and right) were examined. Disposable pre-gelled silver/silver chloride bipolar surface electrodes (rectangular shape, 21x41 mm, 20 mm inter-electrode distance) (F3010, Fiab, Firenze, Italy) were positioned. On each muscle a bipolar
electrode was positioned on the muscular bellies parallel to muscular fibres as follows (Musto et al 2017):

- **MM**: the operator, standing in front of the seated subject, palpated the muscular belly while the subject clenched his/her teeth. The electrodes were fixed parallel to the exocanthion-gonion line and with the upper pole of the electrode under the tragus-labial commissural line.
- **TA**: the muscular belly was palpated during tooth clenching and the electrodes were fixed vertically along the anterior margin of the muscle (corresponding to the fronto-parietal suture).
- **SM**: each electrode was placed parallel to the anterior digastric belly, paramedian to the midline and lightly diverging, 1 cm posterior to the mental symphysis.

A disposable reference electrode was applied to the forehead or on the earlobe (Fig 17). To reduce skin impedance, the skin was carefully cleaned prior to electrode placement and recordings were performed 5 min later, allowing the conductive paste to adequately moisten the skin.

![Figure 17: representation of the electrodes position.](image)

**Electromyography**

Surface EMG activity was recorded using a computerized instrument (Easymyo, 3 Technology S.r.l., Udine, Italy). The analogue sEMG signal was amplified (gain 100, bandwidth 0–1000 Hz, peak-to-peak input range from 0 to 3600 mVpp) using a differential amplifier with a high common mode rejection ratio (CMRR 100 db in the
range 0–60 Hz, input impedance 100Gohm), digitized (24 bit resolution, 4000 Hz A/D sampling frequency), and digitally filtered (high-pass filter set at 30 Hz, low-pass filter set at 400Hz, band-stop for common 50–60 Hz noise). The signals were averaged over 25 ms, with muscle activity assessed as the root mean square (RMS) of the amplitude ($\mu V$). SEMG signals were recorded for further analysis. Before acquisition session, the subjects were properly trained to elicit true teeth maximal voluntary contraction using an on-time sEMG signal visualization.

2.2.4. Measurements

PHASE 1

Each appointment (T1 and T2, with a 2 weeks interval between them), was composed by three acquisition steps:

- **Masticatory muscles standardisation procedures:** two 10-mm thick cotton rolls were positioned on the mandibular second premolars/first molars of each subject, and a 5-s maximum voluntary contraction (MVC) was recorded to standardise TA and MM sEMG signal. The mean sEMG potential obtained in the first acquisition was set at 100%, and all further sEMG potentials were expressed as a percentage of this value ($\mu V/\mu V \times 100$) (Ferrario et al 2000).

- **Submental muscles standardisation procedures:** the subjects were invited to push their tongue at their best (without teeth clenching) against the palate, and a 5-s sEMG SM activity was recorded. All further SM sEMG potentials were expressed as a percentage of this value ($\mu V/\mu V \times 100$). This test was repeated twice (A and B) in each appointment, in order to assess SM muscles standardisation procedures repeatability.

- **Maximal voluntary teeth clenching:** TA, MM and SM sEMG activity was recorded during a 5-s maximal voluntary clenching (MVC) test in intercuspal position (IP); as “the complete intercuspation of the opposing teeth independent of condylar position, sometimes referred to as the best fit of the teeth regardless of the condylar position” (The Academy of Prosthodontics, 2005). The subjects were invited to clench as hard as possible and to maintain the same level of contraction during the entire test.
PHASE 2

Each appointment (T1 and T2, with a 2 weeks interval between them), was composed by four acquisition steps:

- Masticatory muscles standardisation procedures.
- Submental muscles standardisation procedures.
- Maximal voluntary teeth clenching.
- Saliva swallowing: we asked the subjects to swallow their saliva; a 5-s sEMG activity was recorded. They were asked to bring their teeth in contact during swallowing and to keep them in rest position (with no occlusal contacts) at the end of it. We repeated the exercise twice (A and B) in each appointment to assess intra-appointment repeatability. A 90-s break period elapsed between the two acquisitions in order to let the subjects drink some water to wet their mouth and collect new saliva.

For each acquisition the best performance 3s intervals were isolated and analyzed.

During the tests, the subjects were asked to perform at their best, to avoid head and neck movements and maintain a relaxed facial expression to reduce cross-talks. Within subject, test order was randomised by a computer random number generator and 1 minute rest period was allowed. All acquisitions were made by the same operator.

2.2.5. sEMG data analysis

PHASE 1

The instrument software tools analyzed the sEMG waves computing two standardized indexes as follow:

- Muscles symmetry was estimated calculating the percentage overlapping coefficient (POC, %) (Ferrario et al 2000), an index of symmetric muscular contraction ranging between 0% and 100%. When two paired muscles contract with perfect symmetry, a POC of 100% is obtained. Temporalis, Masseter and submental muscles POCs were obtained for each subject for each acquisition session.
- Muscles activity was estimated calculating the standardized activity index (Impact, %). It quantifies the total muscular recruitment during MVC relative to the standardisation test computing the mean total muscle activities as the
integrated areas of the sEMG potentials over time (Ferrario et al 2002). The index was obtained for each muscle and for each acquisition session.

Submental muscles indexes were computed twice in each appointment since standardisation procedures were repeated (T1A-T1B; T2A-T2B).

PHASE 2
Using the instrument software tools, for each acquisition session, sEMG waves were analyzed computing two standardized indexes as follow:

- POC index
- IMPACT index

Other measurements were done by analysing the graphic of the swallowing wave of each muscle:

- Duration of activation of each muscle, considered as the interval in which muscle activity was higher than 10% of its standardization test. 10% value was chosen in order to not consider muscular basal activity.
- Duration of the whole swallowing test, described as the interval between the beginning of first muscle activation and the end of last muscle activation.
- Intensity of the spike and the time of the spike of activation of each muscle relatively to its total duration of activity (Fig18).

Figure 18: example of an electromyographical signal of a muscle activated during swallowing. X axes: time (ms), Y axes: intensity of activation (μV/μVx100).

2.2.6. Statistical Evaluations

PHASE 1
Descriptive statistics were computed for all sEMG indexes for MVC. For the evaluation of the protocol reliability a two-way factorial analysis of variance was made: "Factor 1"
(F1-appointment, T1 vs T2), "Factor 2" (F2- standardisation session, A vs B) and F1 X F2.

Mean absolute difference (MAD), technical error of measurement (TEM) and relative error magnitude (REM) were calculated to quantify the reliability of the proposed ssEMG protocol. MAD is the average of absolute differences between the values of 2 sets of measurements; TEM or Dahlberg’s error was used to evaluate the random error and was computed as

$$\text{TEM} = \sqrt{\frac{\sum D^2}{2n}}$$

where D is the difference between each couple of replicate measurements and n is the number of couples (Rosati et al 2010).

The REM was obtained by dividing the MAD for a variable by the grand mean for that variable and multiplying the result by 100; it represents an estimate of error magnitude relative to the size of the measurement.

To evaluate the effect of sex and the possible differences among masseter, temporalis and submental muscles in muscle symmetry and recruitment during teeth clenching a set of analyses of variance were made. For both POC and Impact indexes, a two-way ANOVA (factor 1: muscles, temporalis, masseter and submental; factor 2: sex) was made. For all statistical tests, significance was set at 5% (P < 0.05), with a beta error (Type II) larger than 0.95 (Sforza et al 2011).

PHASE 2

Descriptive statistics were computed for all sEMG indexes for swallowing. The evaluation of the protocol reliability was similar to the one of phase 1.

To evaluate the effect of sex and the possible differences in muscles symmetry, recruitment and duration of activation during swallowing a set of analyses of variance were made. For both POC and Impact indexes, a two-way ANOVA (factor 1: muscles, temporalis, masseter and submental; factor 2: sex) was made. For all statistical tests, significance was set at 5% (P < 0.05), with a beta error (Type II) larger than 0.95 (Sforza et al 2011).
2.3. Results

PHASE 1

The ssEMG assessment of SM muscles was reliable, as shown in Table 1. For both POC and Impact indexes, no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found, with small MAD and TEM values. In contrast, the REM value of impact index was larger than 30%. Since SM indexes were repeatable for all the acquisitions (T1 A and B; T2 A and B), mean values were computed for the subsequent comparisons (between males and females and among muscles).

Data about masticatory and submental area muscles symmetry and recruitment during teeth clenching are reported in Table 2; the values of the symmetry index POC were larger than 80% for all couples of muscles. Regarding recruitment values, anterior temporalis (TA) and masseter (MM) muscles indexes resulted approximately the same as the ones recorded during the standardisation acquisition (clenching on cotton rolls), while SM developed on average 31% of the activity expressed during the standardisation test (pushing the tongue against the palate). The recruitment of submental area muscles resulted significantly lower than that of MM and TA in both sexes (P<0.001). Side-related differences were not evaluated due to the high POC values; for Impact index comparisons we considered a mean between the Impact values of the right and the left side (the values were almost the same).

<table>
<thead>
<tr>
<th>Submental ssEMG reliability during teeth clenching</th>
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</thead>
<tbody>
<tr>
<td><strong>POC (%)</strong></td>
</tr>
<tr>
<td>mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

| IMPACT (%) | T1 vs T2 | A vs B | MAD | TEM | REM |
| mean | 31.99 | N.S. | N.S. | 11.48 | 10.80 | 37 |
| SD | 14.52 | |

Table 1: standardized surface electromyography (ssEMG) indexes –POC (%) and Impact (%) mean and standard deviation (SD) for Submental Muscles and their reliability, evaluated computing a two-way analysis of variance: F1 (T1 vs T2) and F2 (A vs B). No F1 X F2 interactions. N.S. not significant (p>0.05). Mean Absolute Difference (MAD); Technical Error of Measurement (TEM) and Relative Error Magnitude (REM).
Table 2: Masticatory muscles (Anterior Temporalis, Masseter, Submental muscles) ssEMG during teeth clenching. Symmetry estimated by the Percentage Overlapping Coefficient index (POC%) and recruitment by the Impact (%) index. Comparisons were performed by 2-way ANOVAs. N.S. not significant (p>0.05). Mean with different superscripts (A and B) differ at post hoc test.

**PHASE 2**

The ssEMG assessment of MM, TA, SM muscles was reliable, as shown in Table 3. For all the indexes, POC, Impact, duration of activation of each couple of muscles and duration of the whole exercise, no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found, with small MAD and TEM values. In contrast, the REM value of Impact and duration indexes were close to 30%. Since indexes were repeatable, mean values (among the four acquisitions T1 A and B, T2 A and B) were computed for the subsequent comparisons.

Data about masticatory and submental area muscles during swallowing are reported in Table 4. The values of the symmetry index (POC) were larger or close to 80% for TA and SM, while they were statistically smaller for MM (p<0.001). Regarding recruitment values, the indexes resulted approximately between 22 and 28% of the ones recorded during the standardisation acquisition for all the muscles. The recruitment of each couple of muscles resulted significantly different from the others in both sexes (P<0.001). The MM resulted to be the less recruited, while the TA was the most activated. As it is reported for the TA activation it seemed to be a sex-related difference (men 30.65%, women 21.70%), although statistical analysis did not demonstrate a significance (due to the high standard deviations). However a larger sample is probably needed in order to better assess this data. Also for the duration of activation of each couple of muscles statistical differences were found among all the couples (p<0.001); the MM showed the shortest activation (an average value of approximately 1 s), while
the SM the longest one (an average value of more than 1.5 s). The duration of the whole swallowing was found to be between 1.5 and 2 s.

In Table 5 and 6 the results about the timing of the maximum spike of activation of each couple of muscles are shown; this value is reported as a percentage of the duration of the activation of each couple of muscles. The results showed that all the couples of muscles had their spike of activation between 35.87 and 42.65% of their total duration of activation. The ssEMG graphic assessment of the position of the spike of activation for MM, TA and SM was reliable, as shown in Table 5. For each couple of muscles, no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found, with small MAD, TEM and REM values. Since indexes were repeatable, mean values (among the four acquisitions T1 A and B, T2 A and B) were computed for the subsequent comparisons. No muscle-related differences nor sex ones have been reported (Table 6).

However the results were close to significance for the comparisons among muscles; a larger sample is needed in order to better assess this data. In Table 7 and 8 the results about the intensity of the maximum spike of activation of each couple of muscles are shown. The MM has the lowest spike around 53% of its total activity, while the SM show the highest spike around 73% of its total activity, with the lowest standard deviation (16.26). The ssEMG graphic assessment of the intensity of the spike of activation for MM, TA and SM was reliable, as shown in Table 7. For each couple of muscles, no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found, with small MAD, TEM and REM values. Since indexes were repeatable, mean values (among the four acquisitions T1 A and B, T2 A and B) were computed for the subsequent comparisons. Muscle-related differences has been reported (Table 8). In Figure 19 we drew a graphic representing the model of the muscular recruitment.
Table 3: standardized surface electromyography (ssEMG) indexes during swallowing: POC (%), Impact (%), duration of activation of each couple of muscles (s) (masseter muscles MM, temporalis muscles TA and submental muscles SM), duration of the whole exercise (s), mean and standard deviation (SD) for masseter, temporalis and submental muscles and their reliability, evaluated computing a two-way analysis of variance: F1 (T1 vs T2) and F2 (A vs B). No F1 X F2 interactions. N.S. not significant (p>0.05). Mean Absolute Difference (MAD); Technical Error of Measurement (TEM) and Relative Error Magnitude (REM).
Table 4: masticatory muscles (Anterior Temporalis, Masseter, Submental muscles) ssEMG during swallowing. POC (%), Impact (%), duration of activation of each couple of muscles (s), duration of the whole exercise (s) indexes are shown. Comparisons were performed by 2-way ANOVAs (except of the duration of the exercise index, for which a Student T test has been conducted). N.S. not significant (p>0.05). Mean with different superscripts (A, B and C) differ at post hoc test.

<table>
<thead>
<tr>
<th></th>
<th>POC(%)</th>
<th>Impact(%)</th>
<th>Duration (s)</th>
<th>Duration TOT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Temporalis</td>
<td>79.29ᴬ  5.5</td>
<td>79.85ᴬ  4.43</td>
<td>1.18ᴬ  0.4</td>
<td>1.83  0.44</td>
</tr>
<tr>
<td>Masseter</td>
<td>76.57ᴮ  9.45</td>
<td>74.53ᴮ  8.88</td>
<td>0.99ᴮ  0.38</td>
<td>0.72ᴮ  0.45</td>
</tr>
<tr>
<td>Submental</td>
<td>84.75ᴬ  3.86</td>
<td>81.46ᴬ  8.57</td>
<td>1.6ᶜ  0.5</td>
<td>1.37ᶜ  0.41</td>
</tr>
<tr>
<td>Comparison muscle</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sex</td>
<td>N.S.</td>
<td>sex</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>muscle x sex</td>
<td>N.S.</td>
<td>muscle x sex</td>
<td>N.S.</td>
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</table>

Table 4: masticatory muscles (Anterior Temporalis, Masseter, Submental muscles) ssEMG during swallowing. POC (%), Impact (%), duration of activation of each couple of muscles (s), duration of the whole exercise (s) indexes are shown. Comparisons were performed by 2-way ANOVAs (except of the duration of the exercise index, for which a Student T test has been conducted). N.S. not significant (p>0.05). Mean with different superscripts (A, B and C) differ at post hoc test.
Table 5: graphical spike position during swallowing, calculated as a percentage of the duration of activation of each couple of muscles (masseter muscles MM, temporalis muscles TA, submental muscles SM). Mean and Standard Deviation (SD) have been calculated. The reliability of the position was calculated computing a two-way analysis of variance: F1 (T1 vs T2) and F2 (A vs B). No F1 X F2 interactions. N.S. not significant (p>0.05). Mean Absolute Difference (MAD); Technical Error of Measurement (TEM) and Relative Error Magnitude (REM).

<table>
<thead>
<tr>
<th>肌群</th>
<th>T1VsT2</th>
<th>AVsB</th>
<th>MAD</th>
<th>TEM</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>35.87</td>
<td>N.S.</td>
<td>19</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>MM</td>
<td>36.33</td>
<td>N.S.</td>
<td>18</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>SM</td>
<td>42.65</td>
<td>N.S.</td>
<td>16</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 6: spike position during swallowing evaluated for temporalis, masseter and submental muscles, shown as a percentage of the duration of activation of each couple of muscles. Comparisons were performed by 2-way ANOVAs. N.S. not significant (p>0.05).

<table>
<thead>
<tr>
<th>SPIKE(%)</th>
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</thead>
<tbody>
<tr>
<td>Temporalis</td>
</tr>
<tr>
<td>Masseter</td>
</tr>
<tr>
<td>Submental</td>
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</tbody>
</table>

Table 7: graphical spike intensity during swallowing, calculated of each couple of muscles (masseter muscles MM, temporalis muscles TA, submental muscles SM). Mean and Standard Deviation (SD) have been calculated. The reliability of the position was calculated computing a two-way analysis of variance: F1 (T1 vs T2) and F2 (A vs B). No F1 X F2 interactions. N.S. not significant (p>0.05). Mean Absolute Difference (MAD); Technical Error of Measurement (TEM) and Relative Error Magnitude (REM).

<table>
<thead>
<tr>
<th>肌群</th>
<th>T1VsT2</th>
<th>AVsB</th>
<th>MAD</th>
<th>TEM</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>60.38</td>
<td>N.S.</td>
<td>7.55</td>
<td>4.94</td>
<td>12</td>
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<tr>
<td>MM</td>
<td>53.45</td>
<td>N.S.</td>
<td>8</td>
<td>5.24</td>
<td>15</td>
</tr>
<tr>
<td>SM</td>
<td>73.45</td>
<td>N.S.</td>
<td>9.35</td>
<td>5.97</td>
<td>13</td>
</tr>
</tbody>
</table>

52
2.4. Discussion

Standardized surface electromyographical (ssEMG) protocols during clenching have been largely studied and accepted by the scientific literature as a useful tool to evaluate the superficial masticatory muscles (TA and MM) activity and therefore they entered in the daily clinical practice (Ferrario et al, 2006; Suvinen et al, 2009). On the contrary, the surface electromyographical assessment of submental muscles and the analysis of dynamic oral functions, such as swallowing, is still an open field of research and further scientific approval is needed before clinical applications. The purposes of the current study were therefore firstly to develop a standardized protocol for the assessment of the SM during a simple static task, like MVC (phase 1); secondly to apply the protocol to...
the analysis of the oral phase of swallowing, in order to draw the muscular recruitment model in a sample of healthy subjects (phase 2).

PHASE1
MVC represents the simplest and best described oral task and therefore it represents an ideal condition to test the reliability of the recording protocol for the muscles of the masticatory apparatus. The standardized protocol proposed in the present study demonstrated a high repeatability of the EMG indexes of all the analyzed muscles both intra and inter-appointment for MVC. During MVC both symmetry and recruitment of submental area muscles were found to be reliable. No significant differences were found for symmetry and recruitment indexes of the submental muscles neither within the single appointment nor between the two recording sessions. However the symmetry showed a low variability, while the recruitment index was highly variable (REM about 37%). One possible explanation for this large value can be found in the biomechanics of the analyzed muscles. During maximal voluntary clenching in intercuspal position, the elevator muscles (among which masseter and temporalis) perform their activities between relatively fixed bone ends, although the mandible undergoes a non-negligible deformation caused by elevator muscles stress during clenching (Jiang and Ai 2002). SM have a fixed origin on the mandible which is solidarized to the skull by the contraction of elevator muscles and an insertion on the hyoid bone whose stability is directly linked to mutual coordination with infrahyoid muscles. The elevator muscles must clench as hard as possible; submental muscles instead must "negotiate" their performance with infrahyoid muscles to ensure the stability of the whole pharyngolaryngeal complex. It is plausible that the larger variability of the SM during teeth clenching may be due to this “dynamism in coordination” to assure hyoid positioning. Regarding muscle recruitment in healthy subjects, we can emphasize that the MM and TA perform similarly when clenching either with cotton rolls or in maximal intercuspidation (as amply demonstrated in previous studies) (Botelho et al 2009; Forrester et al 2011; Frongia et al 2013). As expected the floor of the mouth showed a significantly lower activation than masseter and temporalis muscles, since it works mainly as a jaw depressor. However, in our recordings the SM were recruited with an average value of about 31% of the standardising exercise, which let hypothesise a not
negligible co-working role in clenching. Our results are slightly higher than those (about 24%) reported by a previous study (van Willigen et al 1997). The differences in the values could be due to different standardisation exercises (in the previous study the subject was asked to activate the mouth opening muscles with a blocked jaw). This quite high co-activation of the mouth floor during teeth clenching has been proposed as the result of a motor pattern established with the occlusal overload protection purpose in the event of sudden mechanical failure (van Willigen et al 1997).

Regarding muscles symmetry in healthy subjects, our data confirm those of previous studies that reported mean values of asymmetry lower than 17% for all muscles. (Ferrario et al 2000).

PHASE 2

Regarding swallowing, for the aim of the study, we decided to test a “guided” kind of swallowing, asking the patients to keep their teeth in contact while swallowing. The usefulness of sEMG for the analysis of swallowing have already been assessed (Vaiman 2009; Stepp 2012; Yu et al 2013), however standardized protocols are still needed in order to let comparisons among subjects and longitudinal evaluations. As it is reported this new tested protocol was repeatable for all the analyzed indexes, although an high inter-individual variability was detected especially for the Impact and the duration of activation of masseter and anterior temporalis muscles (MM and TA) (REM values between 21 and 27%). This result could be linked with the voluntary action of jaw elevator muscles, which are recruited in the oral phase of swallowing for the stabilization of the mandible. As it is largely explained the first (oral) part of the oro-pharyngeal phase of swallowing is mainly voluntary and it also receives a cortical control. The oral stage is subjected to marked individual variability, whereas the pharyngeal stage is generally more consistent (Lavelle 1988), since it is linked with a triggered reflex. POC index for MM resulted statistically lower than the others. This result is in accordance with other electromyographical studies, conducted with different volumes of the bolus that found an highly asymmetrical pattern of the MM being more influenced by the volume of the swallowed liquid than temporalis and submental muscles. Authors hypothesised that this muscle group was compliant to adaptation and that in a population of healthy subjects it is possible to observe a certain degree of
muscle imbalance, which can be considered as physiological and compatible with normal function. The existence of a preferred chewing side has been demonstrated and it can be said that this imbalance condition can lead to different stimulus between the working and balancing sides during chewing and can contribute to the asymmetric development of the facial skeleton and, consequently, the muscles of this region. The authors speculated that one of the possible explanations for the asymmetry found between the masseter muscle electrical activity may be related to masticatory preference side in the volunteers. However in this study, the normalization was different from the one tested in our protocol, considering (100%) the average of the three repetitions required in the task of maximal voluntary clench as a reference value, when the masseter muscle is at the height of its muscular activity and all other data was analyzed in terms of reference value percentage. In addition the authors did not consider tooth contact and occlusal strength (Pernambuco et al 2011). Higher POC values for SM could be also explained considering the morphological aspects and the electrodes position, since all the mouth floor is composed by muscular overlapping fibers. Activity index (Impact) was statistically different between all the couple of muscles (p<0.001) and the MM showed the lowest activity; this could be explained with a bigger participation of postural and stabilization muscles (TA and SM), instead of strength ones (MM). However the activity of all the muscles was not larger than 30% of their maximal activity. Even the graphical analysis of the spike confirmed our speculation. MM shows the lowest spike of activity with the largest variability, on the contrary SM show the highest spike with a small variability. This could be linked with the reflexive nature of SM action. All the couples of muscles differ for the duration of activation. The MM showed the lowest duration, while the SM showed the longest one. This can be easily understood if it is considered how SM work from the earliest stage until the late portion of the oropharyngeal phase of swallowing. Also the duration of the entire swallowing test is similar to the values demonstrated in previous similar studies (Vaiman et al 2004 b; Wilson et al 2006; Monaco et al 2008). A larger sample of healthy adults is probably needed. A final general consideration has to be conducted. Many parameters showed an high variability between all the subjects and a bigger sample is needed in order to detect swallowing normality values. The normal muscle activity during swallow has several graphic patterns and the results of the quantitative
and qualitative analyses demonstrated that normal swallowing function is highly variable from individual to individual (Vaiman et al 2004 c; Pernambuco et al 2012). A single model of muscular pattern between subjects is impossible to be drawn, but several models have to be done; nevertheless we tried to draw a mean model of recruitment (Fig 19). Each subject showed a repeatability of this model in different acquisition sets. It can be stated that an intra-subject model can be assessed. All our results are in accordance with previous studies. Vaiman et al enrolled about 400 healthy subjects, dividing them into different groups according to their age and asking them to swallow different volume bolus (among these also saliva swallowing has been assessed). They did not give them any indication about teeth clenching. The duration of swallowing was demonstrated to be 1.55 s, SM showed an higher activation than MM and there were not sex related differences. A inter-individual variability has been assessed, with different physiological muscular patterns. (Vaiman et al 2004 a,b,c).

One of the limitation of the present investigation is the standardisation procedure (pushing the tongue against the hard palate) that did not assure to obtain the maximal muscles activity, but it allowed us to identify a repeatable exercise involving a high activity level of submental muscles, which can be used as a reference for subsequent tasks analysis. Another limitation is the reduced number of recruited subjects, since the research was designed as a pilot study aiming at the definition of a new protocol that should be further verified in a larger sample, thus obtaining mean values of the in the population.

2.5. Conclusions

This research represents a preliminary approach for the development of a standardized, simple protocol for the sEMG assessment of the oral phase of swallowing, which could be daily used in the clinical practice. The obtained results have demonstrated the reliability of the protocol; however the high variability between the subjects results in the impossibility of drawing a single model of muscular recruitment for all the subjects. On the contrary in each subject a repeatable muscular model can be seen. The complexity of the task requires a larger sample size for a better assessment of the different physiological models of swallowing. In addition as it is reported this research represents a first step in the electromyographical analysis of swallowing. We evaluated
a para-physiological task, asking the subjects to keep their teeth in contact during swallowing. In the future the protocol could be applied to a “more physiological” kind of swallowing, asking the subjects to swallow in their most comfortable way, without giving them instructions about teeth position. Thus speculations about the activation and the symmetry of masticatory muscles during the oral phase of swallowing in healthy subjects can be done and clinical usefulness can be proved.
3. REFERENCES

Articles


Books