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1A new therapeutic approach for oropharyngeal dysphagia based on pharmacological sensory stimulation with TRP agonists and measurement of salivary neuropeptides as biomarkers for impaired pharyngeal sensitivity

Noemí Tomsen Ferré

A new therapeutic approach for oropharyngeal dysphagia based on pharmacological sensory stimulation with TRP agonists and measurement of salivary neuropeptides as biomarkers for impaired pharyngeal sensitivity

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Director: Prof. Pere Clavé Civit

Co-director: Dr. Omar Ortega Fernández

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Enfermedades Hepáticas y Digestivas

UAB
Universitat Autònoma de Barcelona

INc Institut de Neurociències UAB

PERE CLAVÉ CIVIT, Director of Research and Academic Development and head of the Unitat de Proves Funcionals Digestives of the Hospital de Mataró (Consorci Sanitari del Maresem), Associate Professor of Surgery of the Universitat Autònoma de Barcelona and principal investigator of the CIBERehd group CSdM-UAB.

OMAR ORTEGA FERNÁNDEZ, post-doctoral researcher of the CIBERehd group CSdM-UAB.

State:

The report entitled "A new therapeutic approach for oropharyngeal dysphagia based on pharmacological sensory stimulation with TRP agonists and measurement of salivary neuropeptides as biomarkers for impaired pharyngeal sensitivity" presented by **NOEMÍ TOMSEN FERRÉ** to apply for PhD degree, carried out in the CIBERehd Research Group of the Hospital de Mataró, has been done under our direction and we authorize its presentation to be evaluated by the corresponding tribunal as considering it completed.

For the record, we sign the present in Mataró on October 15, 2021.

Prof. Pere Clavé	**Dr. Omar Ortega**	**Prof. Patrocionio Vergara**	**Noemí Tomsen**
Director	Co-director	Tutor	PhD student

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SCIENTIFIC PRODUCTION

Some of the results obtained during the time period in which this doctoral thesis has been performed were published in indexed scientific journals:

Original articles:

Annex 1:

Tomsen N, Alvarez-Berdugo D, Rofes L, et al. A randomized clinical trial on the acute therapeutic effect of TRPA1 and TRPM8 agonists in patients with oropharyngeal dysphagia. *Neurogastroenterol Motil*. 2020; :1-12. doi:10.1111/nmo.13821.

Annex 2:

Nascimento W, **Tomsen N**, Acedo S, et al. Effect of Aging, Gender and Sensory Stimulation of TRPV1 Receptors with Capsaicin on Spontaneous Swallowing Frequency in Patients with Oropharyngeal Dysphagia: A Proof-of-Concept Study. *Diagnostics (Basel, Switzerland)*. 2021;11(3). doi:10.3390/diagnostics11030461.

Annex 3:

Tomsen N, Ortega O, Rofes L, et al. Acute and subacute effects of oropharyngeal sensory stimulation with TRPV1 agonists in older patients with oropharyngeal dysphagia: a biomechanical and neurophysiological randomized pilot study. *Therap Adv Gastroenterol*. 2019;12:1-13. doi:10.1177/1756284819842043.

Annex 4:

Tomsen N, Ortega O, Nascimento W, Carrión S, Clavé P. Oropharyngeal Dysphagia in Older People is Associated with Reduced Pharyngeal Sensitivity and Low Substance P and CGRP Concentration in Saliva. *Dysphagia*. Published online 2021. doi:10.1007/s00455-021-10248-w.

Book chapters:

Annex 5:

Alvarez-Berdugo D, **Tomsen N**, Clavé P. Sensory Stimulation Treatments for Oropharyngeal Dysphagia. In: Ekberg O. (eds) *Dysphagia. Medical Radiology*. Springer, Cham. 2018. https://doi.org/10.1007/174_2017_166.

Annex 6:

Tomsen N, Clavé P. Pharmacological use of transient receptor potential (TRP) ion channel agonists in neurological disease and aging: effects on swallowing and implications for nutrition. In: Martin CR, Preedy VR, Rajendram R. (eds) *Assessments, Treatments and Modelling in Aging and Neurological Disease*. 2021. https://doi.org/10.1016/B978-0-12-818000-6.00032-9.

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LIST OF ABBREVIATIONS

ASIC: acid-sensing ion channel

AUC: area under the curve

BA: Brodmann area

CAPS: capsaicin

CGRP: calcitonin gene-related peptide

CIT: citral

CIT-ISO: citral-isopulegol

CIN-Zn: cinnamaldehyde-zinc

CN: cranial nerve

CNS: central nervous system

CPG: central pattern generator

DSG: dorsal swallowing group

EAT-10: Eating Assessment Tool-10

EEG: electroencephalogram

ECW: extracellular water

EMG: electromyography

EOD: elderly with oropharyngeal dysphagia

FEES: fiberoptic endoscopic evaluation of swallowing

GPJ: glossopalatal junction

HE: healthy elderly

HRM: high-resolution manometry

HV: healthy volunteer

ICD: International Classification of Diseases

ICW: intracellular water

IP: investigational product

ISLN: internal superior laryngeal nerve

LES: lower esophageal sphincter

L-IX: lingual branch of cranial nerve IX

LN: lingual nerve

LV: laryngeal vestibule

LVC: laryngeal vestibule closure

LVO: laryngeal vestibule opening

MENT: menthol

MMI: minimal massive intervention

MS: modified starch

NTS: nucleus tractus solitarius

Ph-IX: pharyngeal branch of cranial nerve IX

OD: oropharyngeal dysphagia

OSR: oropharyngeal swallow response

PA: phase angle

PAS: penetration-aspiration scale

PIPE: piperine

pMEP: pharyngeal motor evoked potential

PSD: post-stroke dysphagia

pSEP: pharyngeal sensory evoked potential

sLORETA: standarized brain electromagnetic tomography

SP: substance P

SSF: spontaneous swallowing frequency

TMS: transcranial magnetic stimulation

TP: thickening product

TRP: transient receptor potential

TRPA1: transient receptor potential channel subfamily ankyrin member 1

TRPM8: transient receptor potential channel subfamily melastatin member 8

TRPV1: transient receptor potential channel subfamily vaniloid member 1

UES: upper esophageal sphincter

UESO: upper esophageal sphincter opening

VFS: videofluoroscopy

VSG: ventral swallowing group

V-VST: volume-viscosity swallow test

XG: xanthan gum

INDEX

INTRODUCTION

1. Introduction

Swallowing is a physiological event consisting of the progression of the alimentary bolus from the mouth to the stomach. It is a complex and fast process and involves the coordinated action of more than 30 pairs of muscles in the mouth, pharynx, larynx and stomach. In addition, good coordination between the digestive and respiratory systems is needed [1].

Dysphagia is a symptom that appears when this physiological function is impaired due to multiple diseases and co-morbidities, leading to the appearance of difficulties or discomfort when forming or moving the alimentary bolus. We can classify dysphagia into two types according to the impairment localization: oropharyngeal dysphagia and esophageal dysphagia [2].

Oropharyngeal dysphagia (OD) is a very prevalent swallowing disorder in older people and patients with neurological or neurodegenerative diseases [2]. It is recognized by the World Health Organization as a condition that affects the digestive system and it is classified in the International Classification of Diseases (ICD) with the following codes: 787.2 (ICD-9) and R13.1 (ICD-10) [3]. Nevertheless, it is still undervalued and underdiagnosed in most hospitals and medical centres. In addition, current treatment is mainly compensatory which does not improve the swallowing function, making the research of new therapeutic strategies a step forward in the management, therapeutic approach and quality of life of patients.

This doctoral thesis aims to improve the knowledge of OD pathophysiology and to explore the possibility of developing an active pharmacological treatment for OD, to move from compensation to the recovery of swallowing function.

2. Anatomy of swallowing

2.1. Oral cavity

The oral cavity (Figure 1) intervenes in the salivation, taste, mastication and ingestion of food, but has also a main role in phonation and respiration. It extends from the external lips and cheeks to the internal anterior pillars of the fauces and can be anatomically subdivided into two parts: peripheral or oral vestibule, and central or buccal cavity.

The **oral vestibule** is the space located between the lips, the cheeks and the gingivodental arches, and its opening to the outside is through the buccal orifice. The labial seal is ensured by the union of the two lips that form the buccal orifice: superior and inferior lips

The **buccal cavity** is the central space delimited by the gingivodental arches, the palatal vault and the floor of the mouth, and it is connected with the pharynx through the isthmus of the fauces.

- The palatal vault separates the nasopharynx from the oropharynx and is formed by the soft palate, a muscle-membranous septum located in the most posterior part, and the hard palate, composed by maxillary bone, palate bones and some glandular and mucosal layers and occupying the anterior two-thirds parts of the vault. At the posterior part of the palatal vault there is a central extension, called uvula, and four lateral ones called anterior (glossopalatal arches) and posterior (palatopharyngeal arches) pillars.
- The floor of the mouth is composed by the mylohyoid, digastric and geniohyoid muscles and is delimited by the maxillary bone and the hyoid bone. Its main role is hyoid traction.

The **tongue** is a hydrostatic muscle located in the medial part of the floor of the mouth. It is an organ with a great movement capacity, which can be differentiated in movements of retraction, projection and articulation, that allows the formation and propulsion of the alimentary bolus. The tongue is divided by the terminal sulcus into two parts: 1) the root, constituting the anterior part of the oropharynx and locating the food channels in both lateral sites. It is attached to the soft palate and the epiglottis through the palatoglossal arches and the epiglottis glossoepiglottic folds, respectively; and 2) the body, constituting the mobile part of the tongue and occupying almost the entire buccal cavity. The dorsal part of the tongue is covered by specialized mucosa that contains the taste buds. The taste buds can be found in fungiform papillae (scattered throughout the body of the tongue), circumvallate papillae (between 8 and 12 located in the terminal sulcus) or foliate papillae (situated in the sides of the base of the tongue) and are the responsible of perceiving the five basic tastes: sweet, salty, sour, bitter and umami [4].

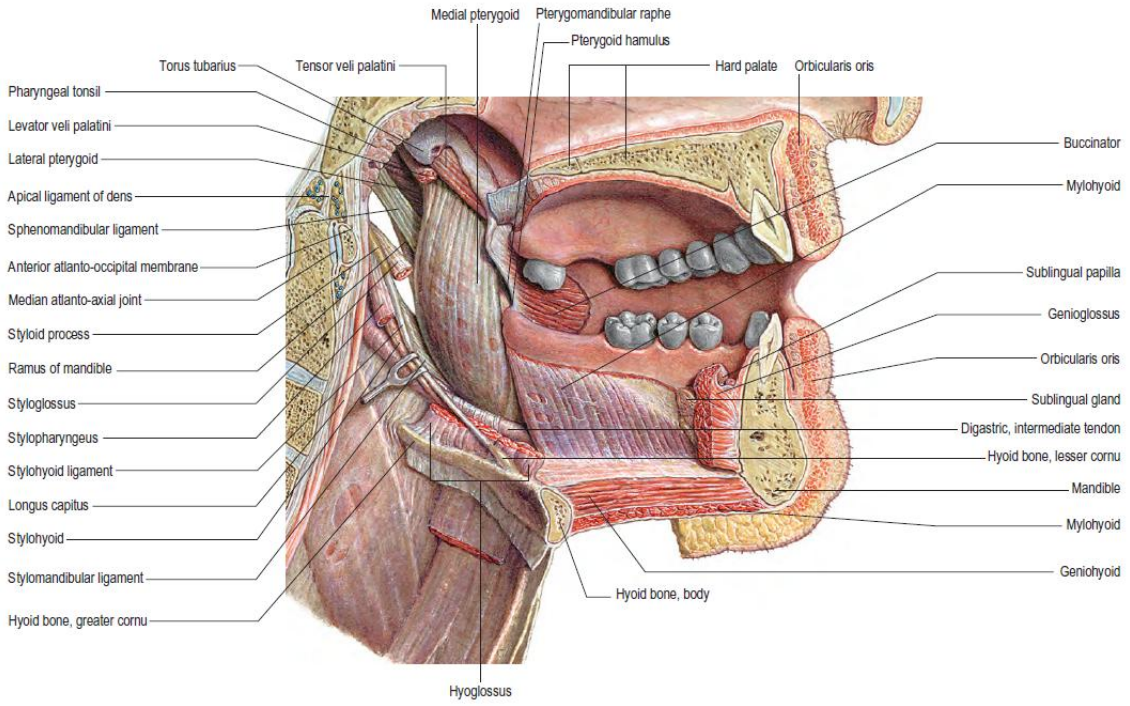

Figure 1: Oral cavity anatomy. Reproduction from Standring et al (2008) [4].

2.2. Pharynx

The pharynx (Figure 2) is the second section of the digestive tract and extends from the posterior part of the nostril and mouth to the larynx, trachea and oesophagus. Physiologically it is a mixed duct as it develops functions of both the digestive and respiratory systems. Anatomically, is divided in three main parts:

1. The superior section is named **nasopharynx**, the main function of which is respiratory and phonatory**.** It extends from the base of the skull, behind the nostril (communicating with it through the coanes) to the soft palate (connecting with the oropharynx through the isthmus of the pharynx). During deglutition, the palate veil raises and contacts the posterior wall of the pharynx forming the velopharyngeal junction, which closes the communication between the oropharynx and the nasopharynx and prevents nasal regurgitation of the alimentary bolus during swallowing.
2. The **oropharynx** is the space between the isthmus of the pharynx at the top and the plane formed by the hyoid bone at the bottom, and communicates with the oral cavity through the isthmus of the fauces. Its function combines both breathing and feeding, allowing the passage of the alimentary bolus towards the digestive tract (through the pharynx) and the air towards the respiratory tract (through the larynx).
3. The lowest section of the pharynx is called **laryngopharynx** or **hypopharynx**. It has a funnel shape and its function is exclusively the passage of food to the oesophagus. It is located

behind and parallel to the larynx and extends from the hyoid bone to the cricoid cartilage, at the sixth cervical vertebra, coinciding with the point where the oesophagus begins. The laryngeal orifice, which has an elliptic or rhomboid shape, is found in the anterior wall between the edges of the epiglottis at the top and the aryepiglottic folds at the bottom. The pyriform sinus is found below the arytenoepiglottic folds, which are an extension of the hypopharynx.

The pharynx is formed by three histological layers: the inner mucosa, the pharyngobasilar fascia (an intermediate fibrous layer with slow-twitch muscles that participate in breathing and the phonation process) and an external muscular layer. This muscular layer is made up of 10 bilateral, striated and fast-twitch muscles that participate in the swallow response: three pairs of constrictor muscles and two of lift muscles. The constrictor or intrinsic muscles (upper, middle and lower constrictor muscle) are formed by transverse and oblique fibres and their function is to narrow the pharynx through peristaltic movements when the bolus passes. The lift or extrinsic muscles (palatopharyngeal, stylopharyngeus and salpingopharyngeal) have the function of elevating and shortening the pharynx during swallowing [5].

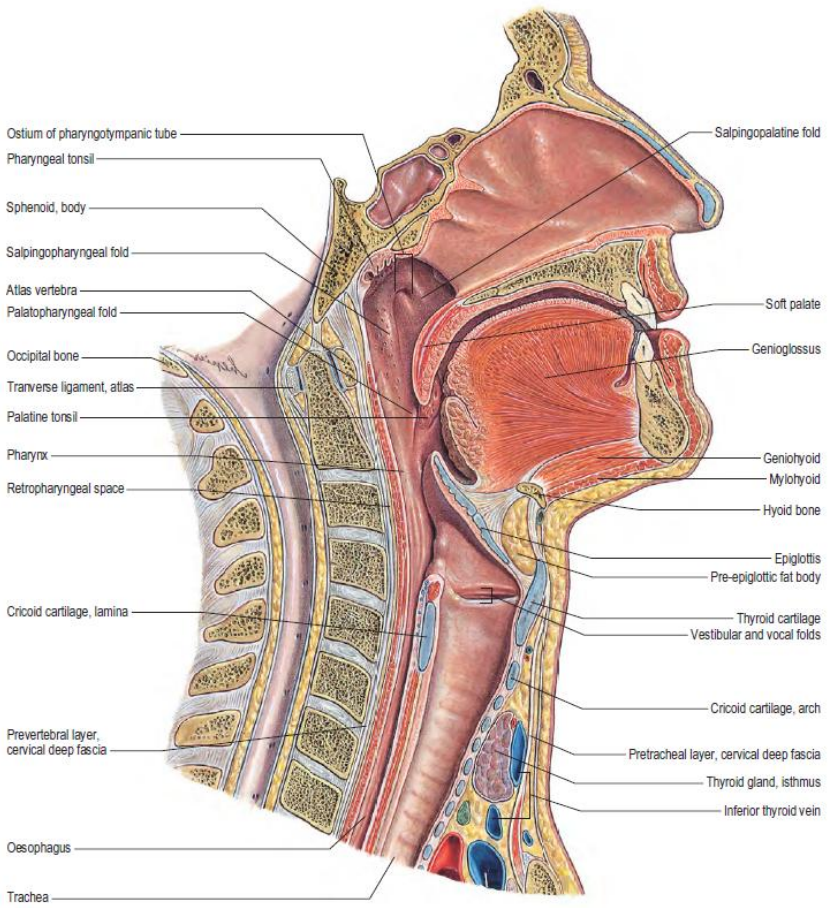

Figure 2: Pharynx anatomy. Reproduction from Standring et al (2008) [5].

2.3. Larynx

The larynx (Figure 3) is part of the respiratory tract and contains the phonation organ. It is located below the hyoid bone and the tongue, in front of the laryngopharynx (communicating cranially), and above the trachea (communicating caudally). It is composed of a cartilaginous skeleton, articulations and ligaments that join the cartilages, the muscles that mobilize them and is lined with respiratory mucosa. The main cartilages are the thyroid, the cricoid (located below the thyroid), the arytenoids (of which there are two resting on the upper edges of the cricoid) and the epiglottis (attached at the bottom of the thyroid via the thyro-epiglottic ligament; its anterior or lingual face is covered by lingual mucosa forming three gloss-epiglottic folds delimiting the valves). During deglutition, the base of the epiglottis rises and moves forward through the contraction of the aryepiglottic muscle, pressure from the base of the tongue and the displacement of the hyoid bone upward and forward. This movement closes the airway and deflects the bolus to the hypopharynx, a very important process for the protection of the respiratory airway during swallowing to avoid respiratory complications.

Internally, the larynx is divided in three parts:

- **Laryngeal vestibule (LV)**: space that constitutes the entrance to the larynx. It ends in the vestibular folds or false vocal cords.
- **Laryngeal ventricle**: space limited by the vestibular folds (upper part) and by the true vocal cords or vocal folds (lower part).
- **Infraglottic cavity**: space between the vocal cords and the trachea [6].

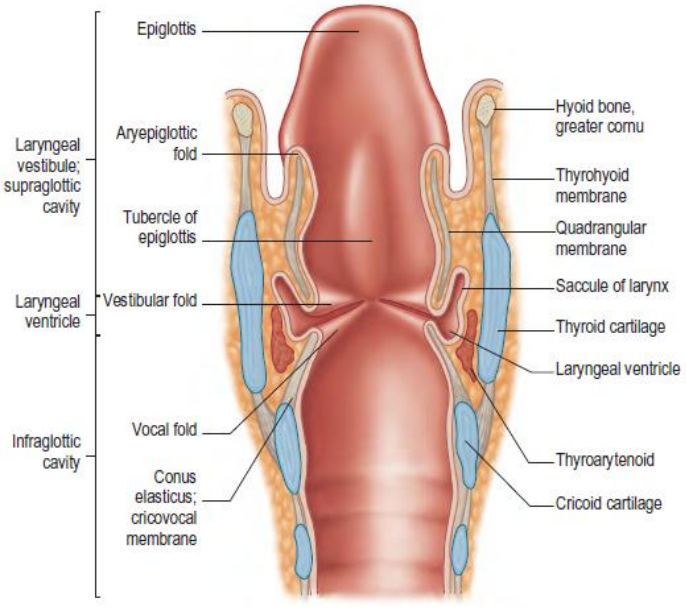

Figure 3: Larynx anatomy. Reproduction from Standring et al (2008) [6].

2.4. Esophagus

The oesophagus is the muscular tube that communicates the pharynx with the stomach. The composition of the muscular layer changes along the structure: the first 5-7cm are formed entirely by striated muscles of involuntary activation and, as it advances towards the stomach, the proportion of smooth muscle increases in the transition zone until it becomes the only type found in the mid and distal oesophagus. The contraction of these muscles causes peristaltic waves that conduct the alimentary bolus to the stomach.

There are two well-differentiated valves:

- **Upper esophageal sphincter (UES):** this is a high pressure zone that separates the pharynx from the oesophagus. It measures 2-4 cm and is located at the fifth or sixth cervical vertebra. The UES is formed by the cricopharyngeal muscle, the lower part of the pharyngeal constrictor muscle and the upper part of the cervical oesophagus muscle. Under physiological conditions, it is tonically closed to prevent the passage of air into the digestive tract or reflux from the oesophagus to the pharynx.
- **Lower esophageal sphincter (LES):** this consists of smooth muscle composed of two semicircles of circular fibers: a) sling muscle fibers, located at the level of the greater curvature of the stomach; and b) short clasp fibers, at the level of the lesser curvature of the stomach. This structure has a basal pressure at rest, which relaxes when a peristaltic wave occurs in the esophageal body, with gastric and esophageal distension on the passage of the bolus [7]–[10].

3. Biomechanics of swallowing

The superior aerodigestive tract has two basic functions: breathing and swallowing. The oropharyngeal swallow response (OSR) requires the coordinated movement of the several oropharyngeal structures, which reconfigure from respiratory to digestive tract during deglutition, allowing the transfer of the bolus from the mouth to the oesophagus and then recover the respiratory configuration again [11], [12]. This complex response has three sequential phases [13]–[15]:

3.1. Oral phase

The oral phase (Figure 4a) is under voluntary control and divided into two sub-phases:

- **Oral preparatory phase**: its main function is the formation of the alimentary bolus. It is characterised by voluntary actions such as mastication. During this sub-phase, the jaw and the anterior part of the tongue go down and the lips separate, increasing the volume of the oral cavity and allowing the introduction of food. Through mastication, which is characterised by cyclic mandibular movements synchronised with the transport of food to the molars by the tongue and cheeks, solid foods are disintegrated and minced by the action of the denture. In addition, the saliva has a main role in hydration, lubrication, formation of the bolus and initiation of digestion with the contribution of salivary α-amylase [16].
- **Oral propulsive phase**: its main function is the propulsion of the bolus from the oral cavity to the oropharynx. The anterior part of the tongue presses the hard palate positioning the bolus in the posterior part of the oral cavity. At the same time, the posterior part of the tongue is in contact with the soft palate, forming the glossopalatal junction (GPJ), in order to avoid the early entrance of the bolus to the oropharynx. When the propulsion starts, the tongue presses the hard palate and generates an anteroposterior pressure wave that propels the bolus to the oropharynx. At this moment, the soft palate is elevated in order to open the GPJ and to close the nasopharynx [17].

The oral phase is finalized when the last portion of the bolus enters the oropharynx and the posterior part of the tongue closes against the GPJ in order to avoid the bolus re-entering the oral cavity [17].

3.2. Pharyngeal phase

This takes place once the oral phase is completed, although it may be initiated in response to pharyngeal stimuli, and it is an involuntary response. This phase includes the OSR and begins with the entrance of the bolus into the pharynx and ends with its exit through the UES. It starts with the stimulation of the pharyngeal receptors which send the sensory information to the central nervous system (CNS) to initiate the OSR. The biomechanical changes of the OSR bring about the temporal configuration of the oropharyngeal structures changing from the respiratory to the digestive tract, the transference of the bolus from the mouth to the oesophagus and the recovery of the respiratory configuration. All these changes are produced through the coordinated opening and closing of the four main valves involved in deglutition: the GPJ, the velopharyngeal junction, the LV and the UES (Figure 4b).

The pharyngeal phase starts with the elevation of the soft palate (GPJ opening) and the upward movement of the posterior pharyngeal wall to close the nasopharynx (velopharyngeal junction closure) to avoid the regurgitation of the bolus through the nose. At the same time, the vocal cords and arytenoids are adducted, closing the airway. In addition, the arytenoids bring the base of the epiglottis closer. Then, a retroflexion of the epiglottis occurs in response to passive pressure from the base of the tongue and the active contraction of the aryepiglottic muscles, completing the closure of the LV (LVC) and avoiding the entrance of the bolus to the larynx. The LVC is the main seal that protects the entrance of material into the laryngeal vestibule and the closure of the vocal folds is the main mechanism protecting the aspiration of material into the airway.

At the same time, the hyoid and the larynx move upward and anteriorly due to the suprahyoid and pharynx longitudinal muscles, positioning the entrance of the larynx below the base of the tongue in order to better protect the respiratory airway. All these actions also allow the shortening and expansion of the hypopharyngeal space and the UES opening (UESO) and elevation to enable the pass of the bolus to the esophagus. In addition, the propagated pharyngeal contraction has a minimal role in the propulsion of the bolus but facilitates pharyngeal clearance and works in concert with pharyngeal shortening to minimize hypopharyngeal residue [18]–[21].

3.3. Esophageal phase

This phase starts with the opening of the UES and the passage of the bolus through it. The UES opening depends on four main mechanisms: 1) the interruption of the vagal tone of the cricopharyngeal muscle which allows the relaxation of the muscle contraction that kept it closed; 2) the traction on the anterior side of the sphincter by the suprahyoid muscle contraction; 3) the pressure on the sphincter exerted by the alimentary bolus propulsion forces (process that directly depends on the lingual propulsion force); and 4) the sphincter compliance that allows its complete relaxation, with low residual pressures and limited resistance during the passage of the bolus [22]. Peristalsis in the upper third is controlled by sequential activation of motor neurons in the CNS, while peristalsis in the mid esophagus and LES relaxation is controlled by enteric motor neurons in the myenteric plexus. Main inhibitory neurotransmitters are nitric oxide, vasoactive intestinal peptide, ATP, Pituitary adenylate cyclase-activating polypeptide and calcitonin gene-related peptide (CGRP) and main excitatory neurotransmitters are acetylcholine and tachykinins (Figure 4c) [23].

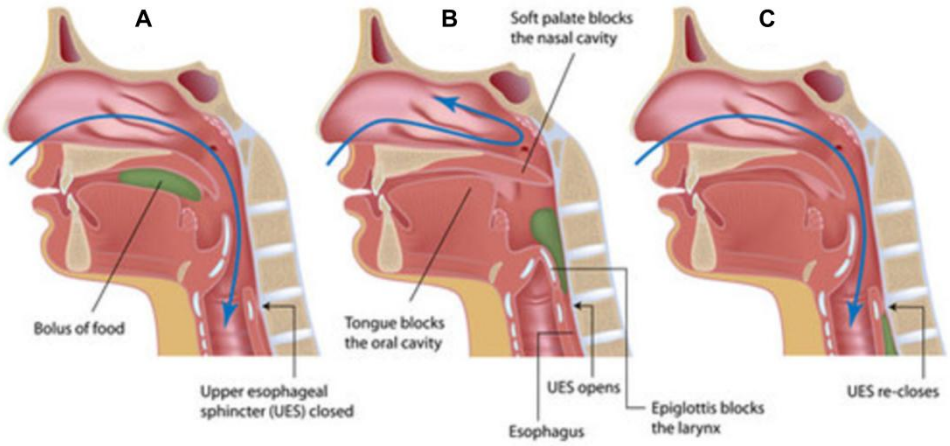

Figure 4: Side view of head and neck during swallowing: a) oral phase; b) pharyngeal phase; c) esophageal phase. Reproduction from Humbert et al (2015) [24].

4. Neurophysiology of swallowing

The proper generation of a coordinated OSR requires the interaction and connection of several areas and elements from the CNS and the peripheral nervous system. It also requires structural integrity of the oropharynx and larynx, proper function of 30 pairs of muscles and coordination with the respiratory system.

Stimuli from the alimentary bolus are perceived by the peripheral sensory receptors during deglutition; this information is sent by the afferent nerves (cranial nerves I, V, VII, IX and X) to the central pattern generator (CPG) located in the medulla oblongata of the brainstem, and to the somatosensory cortex and subcortical structures such as the amygdalae and the basal ganglia [2].

The voluntary control of the oral phase and part of the pharyngeal phase of deglutition are conducted in cortical areas of the brain such as the precentral and inferior frontal gyri as well as other adjacent cortical areas of the sylvian fissure and the lateral and precentral cortex[14]. In healthy individuals, deglutition activates these cortical areas in a bilateral but asymmetric fashion, which implies the existence of a dominant hemisphere [25], [26].

The OSR of the pharyngeal phase of deglutition is produced in the CPG found in the medulla oblongata of the brainstem. The CPG is composed of two well-communicated groups of interneurons: the dorsal swallowing group (DSG) and the ventral swallowing group (VSG). The DSG is found in the nucleus tractus solitarius (NTS) within the medulla oblongata and integrates the afferent information from the peripheral nerves and the modulating signals

coming from cortical and subcortical structures to generate the swallowing motor pattern. The VSG is found in the ventrolateral aspect of the medulla oblongata above the nucleus ambiguus and, upon activation by the DSG, distributes the swallowing motor pattern among the different motor nuclei [11].

The motor nuclei controlling the deglutition muscles are found in the pons of the brainstem (trigeminal motor nucleus and facial nucleus), in the medulla oblongata (nucleus ambiguus and hypoglossal nucleus) and in the cervical spinal cord (C1-C2). Motor neurons innervating oropharyngeal muscles project from these nuclei through the cranial nerves V, VII, IX, X, XI and XII and the cervical spinal nerves (C1, C2 and C3) composing the cervical plexus.

The muscles that participate in deglutition are the masticatory, the lingual, the soft palate, the pharyngeal, the laryngeal, the suprahyoid and the infrahyoid muscles. During the oral phase, the masticatory muscles are innervated by the cranial nerves V and VII and the lingual muscles are innervated by the cranial nerve XII. During the pharyngeal phase, the soft palate, the pharyngeal and the extrinsic laryngeal muscles are innervated by the pharyngeal plexus composed by the cranial nerves IX and X, the suprahyoid muscles are innervated by the cranial nerves V, VII and XII, the intrinsic laryngeal muscles are innervated by the recurrent laryngeal nerve (cranial nerve X) and the infrahyoid muscles are innervated by the cervical plexus.

4.1. Sensory innervation of the oropharynx and larynx

The human oropharynx and larynx are innervated by afferent fibres of the lingual branch of the cranial nerves V and IX, the chorda tympani of the cranial nerve VII, the pharyngeal branch of the cranial nerves IX and X and the laryngeal branch of the cranial nerve X.

The tongue is innervated by afferent fibres of the lingual branches of the cranial nerves V, VII and IX (L-IX). The base of the tongue and the circumvallate and foliate papillae are innervated by two of the four major subdivisions of the L-IX. The subdivision of the L-IX that innervates the base of the tongue mucosa is divided into tertiary branches and twigs that run towards the medial axis of the tongue where they meet with the fibres coming from the opposite side. The subdivision of the L-IX that innervates the papillae is divided into lateral and medial branches; lateral branches innervate the foliate papillae and some of the lateral circumvallate papillae, medial branches ipsilaterally innervate most of the circumvallate papillae. The central circumvallate papilla is innervated by fibres coming from medial branches from both sides [27]. The two anterior thirds of the tongue are innervated by the lingual nerve (LN) composed of the lingual branch of the cranial nerve V and some afferent fibres from the chorda tympani. The LN

penetrates the tongue anterior to the circumvallate papillae and subdivides into medial and lateral branches; the medial branches innervate the ventrolateral mucosa of the tongue and the lateral branches innervate the lateral part of the tongue and its tip [28].

The oropharyngeal mucosa is innervated by afferent fibres of the lingual (L-IX) and pharyngeal (Ph-IX) branches of the cranial nerve IX and the pharyngeal branch of the cranial nerve X (Ph-X). The mucosa of the tonsil and peritonsillar area and the lingual surface of the epiglottis are innervated by two of the four subdivisions of the L-IX. The subdivision of the L-IX that innervates the tonsil and peritonsillar area is split into two or three tertiary branches that surround the tonsil. The subdivision of the L-IX that innervates the epiglottis is also split into two or three tertiary branches; one of them innervates the mucosa of the lingual surface of the epiglottis and the others form anastomosis with afferent fibres from the laryngeal sensory branch of the cranial nerve X, the internal superior laryngeal nerve (ISLN). The lateral and posterior walls of the pharynx, including the pharyngopalatine arch, are innervated by the three subdivisions of the Ph-IX together with the afferent fibres of the Ph-X; all these afferent fibres form a dense nervous plexus in the mucosa of this region [27].

The mucosa of the hypopharynx and larynx is innervated by the ISLN, which penetrates the thyrohyoid membrane and splits into three branches. The laryngeal surface of the epiglottis is innervated by the superior branch of the ISLN, which penetrates the epiglottis through the aryepiglottic fold and subdivides into twigs that form a dense network with the fibres arriving from both sides. The aryepiglottic fold, the laryngeal vestibule and the vocal folds are innervated by the middle branch of the ISLN, which splits into interconnected twigs that form a network under the mucosa. The other parts of the hypopharynx are innervated by the inferior branch of the ISLN, the one with greatest diameter and most complex distribution: the superior subdivision of which innervates the arytenoid cartilage, the middle subdivision innervates the region posterior to the arytenoids and the interarytenoid muscles and the inferior subdivision innervates the mucosa over the anterior wall of the hypopharynx (Figure 5) [29], [30].

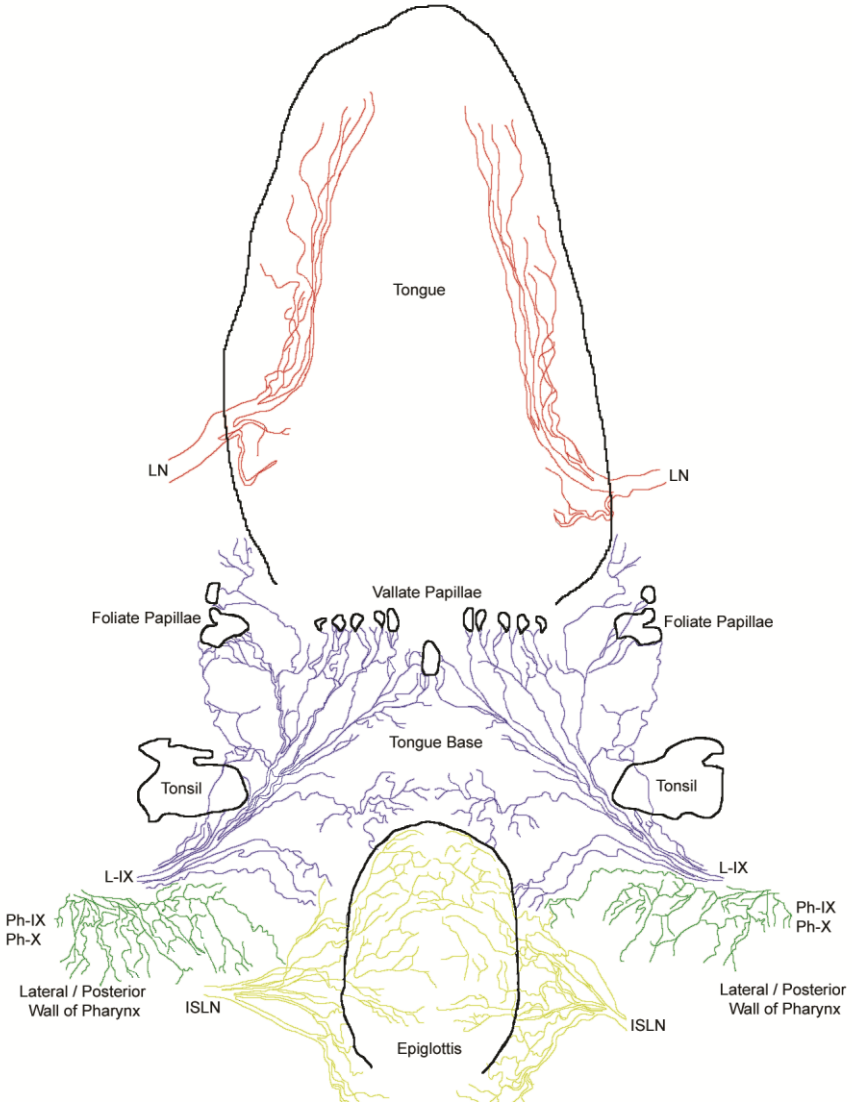

Figure 5: Schematic representation of oropharynx innervation: Lingual nerve (LN) in red; Lingual branch of glossopharyngeal nerve (L-IX) in blue; pharyngeal plexus (Ph-IX and Ph-X) in green; Internal superior laryngeal nerve (ISLN) in yellow. Reproduced from Alvarez-Berdugo et al (2016) [31].

4.2. Anatomy and histology of the afferent (sensory) pathway

There are several structures and receptors located in the oropharyngeal mucosa that have an important role in the integration of different sensory stimuli (such as chemical, mechanical and thermal). Initiation and modulation of swallowing requires sensory feedback from the afferent stimuli from the alimentary bolus and its physicochemical characteristics. This sensory information will help in several processes such as the bolus preparation and the initiation and regulation of the OSR.

- **Taste buds:** Taste buds (Figure 6f) distinguish between the five basic tastes: salt, sweet, bitter, sour and umami. They are found on the fungiform papillae of the two

anterior thirds of the tongue and the soft palate, in the rifts of the circumvallate papillae and on the foliate papillae of the tongue base. Taste buds are onion-like structures formed by specialized epithelial cells [32]–[34], which can be classified according to their morphology and function as Type I, II, and III. Type I cells are the most abundant (50-75% of the taste bud cells) and provide cellular support to the other cells; they also transduce the salt taste [35], [36]. Type II cells transduce the sweet, bitter and umami tastes, depending on the specialized receptor they express [37], [38]. Type III cells establish a synaptic union with the nerve endings that supply the taste buds and transduce salt and sour tastes [38], [39]. All these taste bud cell types are connected; Type I cells provide support to the perception and synaptic communication of Type II and III cells. Type II cells finely perceive tastes and send signals to Type III cells that will transmit the stimulus information to the nerve endings [35]. The nerve fibres supplying the taste buds penetrate the basal lamina of the epithelium and form protrusions that surround Type III cells to establish synapse [39], [40].

- **Smell:** The smell of volatile particles of the alimentary bolus is released in the posterior part of the oral cavity. These particles can produce a retronasal stimulation of the receptors located in the olfactory epithelium of the upper part of the nasal cavity and are transmitted to the olfactory bulb [41], [42].
- **Free Nerve Endings:** The free nerve endings (Figure 6e) are located below the basal lamina and, to a lesser extent, penetrating the epithelium. They are generally Aδ and C type and have the role of perceiving painful, chemical and thermal stimuli [43]–[45].
- **Mechanoreceptors and other structures:**
    - **Maisner corpuscles**: Meissner corpuscles (Figure 6a) perceive painless light mechanical stimuli. They are found in groups within the papillae disposed in different directions. They are supplied by one to three myelinated nerve fibres that lose their myelin sheath on entering the corpuscle and then divide into branches or form a discoid shape. The nerve endings are covered with several lamellar layers separated by collagen fibre filled interspaces. The corpuscles are covered with a collagen capsule that keeps them separated from the basal lamina of the epithelium [46]–[48].
    - **Merkel cells**: Merkel cells (Figure 6b) perceive the lightest touch without pain. They are found alone or in groups in the basal layer of the epithelium, always bound to the basal lamina. They are innervated by nerve endings that penetrate the basal lamina after losing their myelin sheath and surround the

base of these cells. Merkel cells present granules in the basilar end and protrusions of cytoplasm that contact neighbouring epithelial cells at the apical end [43], [49]–[51].

- **Pacini Corpuscles**: Pacini corpuscles (Figure 6c) perceive deep mechanical stimuli without pain. They are found in the deepest layer of the submucosa. They are innervated by a single myelinated nerve end that penetrates the corpuscle in a straight line. Pacini corpuscles are lamellar structures [52], [53].
- **Krause Bulbs**: Krause bulbs (Figure 6d) perceive light touch and cold stimuli. They are found within the papillae. They are innervated by one to three myelinated or not myelinated nerve endings, when both kinds of nerve endings penetrate the same bulb they never contact each other. Once the nerve endings have penetrated the bulb, they run in coils and split into several axon terminals. Krause bulbs are covered by a capsule composed of fibroblasts and collagen fibres [54], [55].

- **Chemosensing**: The CN V, IX and X nerves innervate the more effective areas that induce the OSR and project to the CPG and cortical and sub-cortical structures. These afferences express the main molecular targets responsible for the somatosensorial stimuli integration: the transient receptor potential channel (TRP) [41], [42], [56].

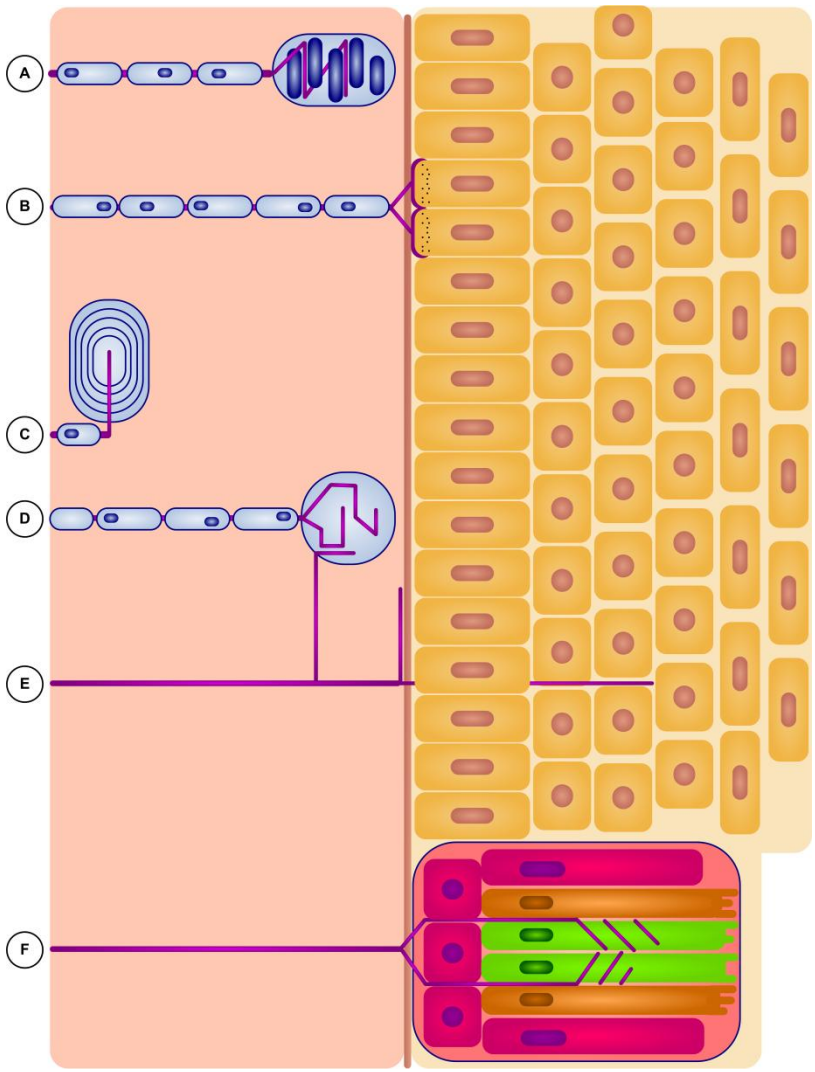

Figure 6: Schematic representation of the oropharynx sensory structures: a) Meissner corpuscle; b) Merkel cell; c) Pacini corpuscle; d) Kraus corpuscle; e) free nerve ending; f) taste bud. Reproduced from Alvarez-Berdugo et al (2016) [31].

4.2.1. Localization and expression of transient receptor potential channels and acid-sensing ion channels in the human oropharynx.

In recent years a group of studies was developed to improve evidence on the localization and expression of TRP receptors in the human oropharynx [57], [58]. The main molecular receptors found in the oropharyngeal mucosa are TRP and acid-sensing ion channels (ASIC) and are located in epithelial cells and sensory afferent fibers with specific patterns of location and expression. These receptors have the capacity to perceive a wide spectrum of sensory stimuli and initiate the transmission of the sensory input from the periphery to the CNS.

There are three types of TRP receptors in the human oropharynx (Figure 7):

- **TRPV1**: The subfamily vaniloid member 1 (TRPV1) was the first TRP receptor identified [59]. It has a homotetrameric structure where every subunit is formed by 6 transmembrane domains, two of which form the pore and the other four form the vanilloid binding site. Both C- and N-terminals are located in the cytosolic space, allowing the specific protein-protein interactions due to the presence of four to six ankyrin repeats. These specific interactions are responsible for the regulation of the channel function, the main regulatory processes of which are the intracellular calcium levels (by the interaction with calmodulin), the PIP2 (by inhibiting the activation) and the PKA and PKC phosphatases (sensitizing the receptor through phosphorylation) [60]–[63]. TRPV1 is mainly expressed in the afferent sensory fibres Aδ and C types and found in several nerve and non-nerve tissues. In the human oropharyngeal mucosa, it is found in greater amounts in the most superficial layers of the epithelial mucosa (specifically in the plasmatic membrane of the epithelial cells), decreasing through the intermediate layer cells to the basal lamina. It is also located in the fibres innervating the mucosa and in some leukocytes located in the submucosa layer. In addition, it is expressed in the lingual mucosa (specifically in the filiform papillae) [57]. TRPV1 receptors are activated by harmful stimuli like temperatures above 43ºC, acid solution with a pH lower than 5.5, and endogenous (anandamide, nitric oxide and hydrogen sulphide) and exogenous chemical compounds (capsaicin, allicin, camphor, cannabidiol and cannabigerol, eugenol, gingerol, piperine and polygodial) [64]. Its activation through the effect of its agonists stimulates the release of different neuropeptides, like Substance P (SP) and CGRP, which produce a local effect through both paracrine and autocrine mechanisms. These neuropeptides trigger the sensitization of the receptor and decrease its activation threshold. SP is known to facilitate the OSR in the larynx through the activation of primary sensory neurons by its direct effect on sensory terminal nerves or the action of pro-inflammatory substances, in addition to being related to the spontaneous swallowing frequency (SSF) of patients with post-stroke OD [65]. SP and CGRP also have a major role in the stimulation of cough reflex through the activation of mechanoreceptor fibres, facilitating the transmission of the sensory input to the sensory cortex and the solitary tract nucleus [66]–[69].
- **TRPA1**: following the description of TRPV1, the subfamily ankyrin member 1 (TRPA1) was identified, also called ANKTM1. Its structure is characterised by being homotetrameric where each subunit has six transmembrane domains. It differs from TRPV1 receptor because only one transmembrane domain forms the pore and its N-terminal end has a spring shape due to its seventeen ankyrin repeats [70]. Within the

human oropharyngeal mucosa, TRPA1 is expressed in the sensory nerve fibres located below the basal lamina and in those which penetrate the epithelium or innervate the blood vessels that irrigate the submucosa. It is also found in specialised structures (such as Langerhans cells) of the lingual mucosa, the mucosa of the epiglottis lingual side and the pharyngeal mucosa intermediate layers [57]. TRPA1 is sensitised by noxious cold temperatures (below 18ºC) and natural (nitric oxide, hydrogen sulphide, bradykinin) or synthetic irritant substances (piperine, 1,4-dihydropyridine, allicin, allyl isothiocyanate, cannabidiol, cannabigerol, cannabichromene, tetrahydrocannabinol, capsiate, cinnamaldehyde, citral, curcumine, eugenol, gingerol, icilin) [71]–[73]. Little is known about its relationship with neuropeptides, but TRPA1 has been described to co-localize with TRPV1, SP and CGRP in a subpopulation of sensory nerves (C-fibres type) from the trigeminal, nodose and dorsal root ganglia [74], [75].

- **TRPM8**: the subfamily melastatin member 8 (TRPM8) is the latest TRP receptor found in the human mucosa. Its structure is also a homotetramer where the subunits have six transmembrane domains: two forming the pore while the others act as the binding site of its agonists and as a voltage sensor. The most distinguished characteristic of TRPM8 structure is the cytosolic domain, composed by the N and C terminal domains and its interfacial interactions [76]. Regarding its expression in the human oropharynx, it is mainly found in the nerve fibres innervating the surface mucosa of the oropharynx, tongue and lingual side of epiglottis. These fibres are generally found below the epithelium (forming bundles or individually) but can also be located penetrating the deeper layer of the epithelium or innervating the blood vessels that irrigate the submucosa [58]. TRPM8 activation depends on non-harmful cold stimuli (temperatures between 15 and 30ºC) and refreshing chemical substances (menthol, eucalyptol, isopulegol, geraniol, icilin and linalool). Regarding its relationship with the neuropeptides, it is only known that TRPM8 does not co-localize with TRPV1 and CGRP in the sensory nerve fibres [77].

Regarding the ASIC receptors, it is known that their active structure consists of the association of three subunits, which are characterized by having two transmembrane domains separated by a large, cysteine-rich region in the extracellular domain and with the N- and C- terminals in the cytosolic domain. Their kinetics and gating characteristics depend on whether they are homomeric (uniform subunits) or heteromeric (varied subunits). They are activated against acidic pH which causes a rapid increase of proton concentration in the extracellular domain. All of them are permeable to monovalent cations and protons ($Na^+ > K^+$), some isoforms of

ASIC1 also being permeable to divalent cations, such as calcium [78], [79]. In the human oropharynx two types are localized: a) **ASIC1**: This receptor is found only in the tongue muscles below the mucosa, co-localizing with the nerve fibers that innervate it; and b) **ASIC3**: located at a lower intensity than TRPM8 in the nerve fibers that are below the epithelium and the basal lamina of the tongue, pharynx and epiglottis. It is also found in the nerve fibers that innervate the blood vessels of the tongue (Figure 7) [58].

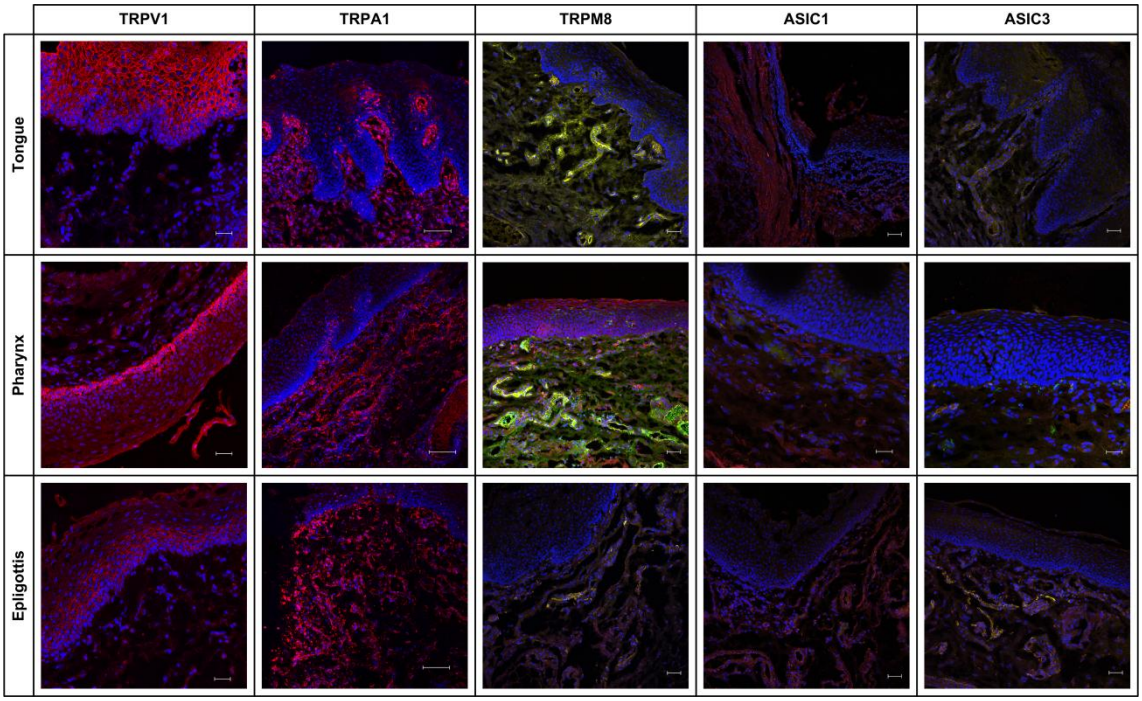

Figure 7: Tongue, pharynx and epiglottis immunofluorescence staining microscopic images. Nuclei are marked in blue (DAPI), specific staining for TRPV1, TRPA1, TRPM8, ASIC1 and ASIC3 are marked in red, and neuron specific enolase A is marked in green. Adapted from Alvarez-Berdugo et al (2016, 2018) [57], [58].

4.3. Central nervous system. Swallowing centre

Once the afferent pathway sends the sensory input to the swallowing centre, the integration and recognition of sensory stimuli take place in order to coordinate the OSR (Figure 8).

The CPG, also called swallowing centre, has the main role to trigger both the spontaneous swallows and the pharyngeal phase of voluntary swallows. It is located in the spinal bulb of the brainstem and is composed by two groups of well communicated interneurons:

- **Dorsal Swallowing Group (DSG)**: located in the NTS. Its synaptic response is characterised by having a very short and stable latency (1-2 ms), indicating that there is a monosynaptic connection pathway with the afferent neurons. Its role seems to be the integration of the information that arrives from the periphery and the cortical

areas, initiating the swallowing motor pattern when the stimuli threshold is reached [11].

- **Ventral Swallowing Group (VSG)**: This is found in the ventrolateral side of the spinal bulb, above the ambiguus nucleus. Its synaptic response needs several pulses in order to be initiated (latency of 7-12 ms), suggesting a polysynaptic pathway. Its activation seems to be through the DSG and its role would be the distribution of the response to the different motor nuclei [11].

4.4. Cortical and subcortical structures

The involuntary control of swallow response and SSF is through bulbar mechanisms. However, the brain cortex has an important role in the voluntary start and modulation of OSR and SSF (Figure 8). The brain areas implicated in the voluntary deglutition process are determined through electrophysiological and neuroimaging studies depending on which main regulatory process is involved (Figure 9):

- Related to taste sensory stimuli processing and perception in the mouth and pharynx: lateral precentral gyrus (that includes the primary motor cortex), lateral postcentral gyrus (that includes the somatosensory cortex), insula and inferior frontal gyrus or Broca's area.
- Related to the attention and planning of swallowing movements: premotor cortex, precuneus, supplementary motor area and anterior cingulate gyrus.
- Probably related to deglutition processing and the integration of taste stimuli: transverse, superior and medial temporal gyrus [25], [80], [81].

In addition, taking into account the motor cortex, it is known that there is a deglutition dominant hemisphere (independent of the dominant hand), since it has been observed that the neural network involved in the swallowing process has a bilateral but asymmetric activation [25], [26] while our studies clearly found the sensory cortex is bilateral and symmetric [82].

Regarding subcortical structures, their specific function on swallowing is still unknown. However, it is suggested that basal ganglia, amygdale, thalamus and cerebellum are involved in the swallowing process in humans.

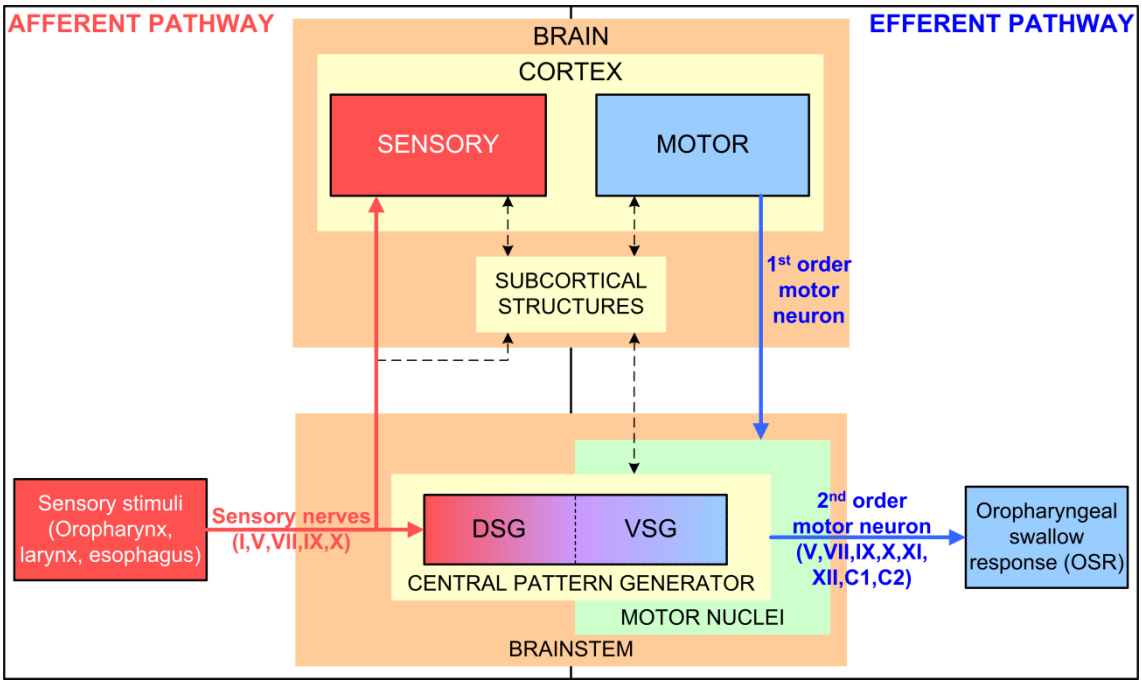

Figure 8: Swallow neurophysiology. DSG: Dorsal swallowing group; VSG: ventral swallowing group. Reproduced from Alvarez-Berdugo et al. 2018 [83].

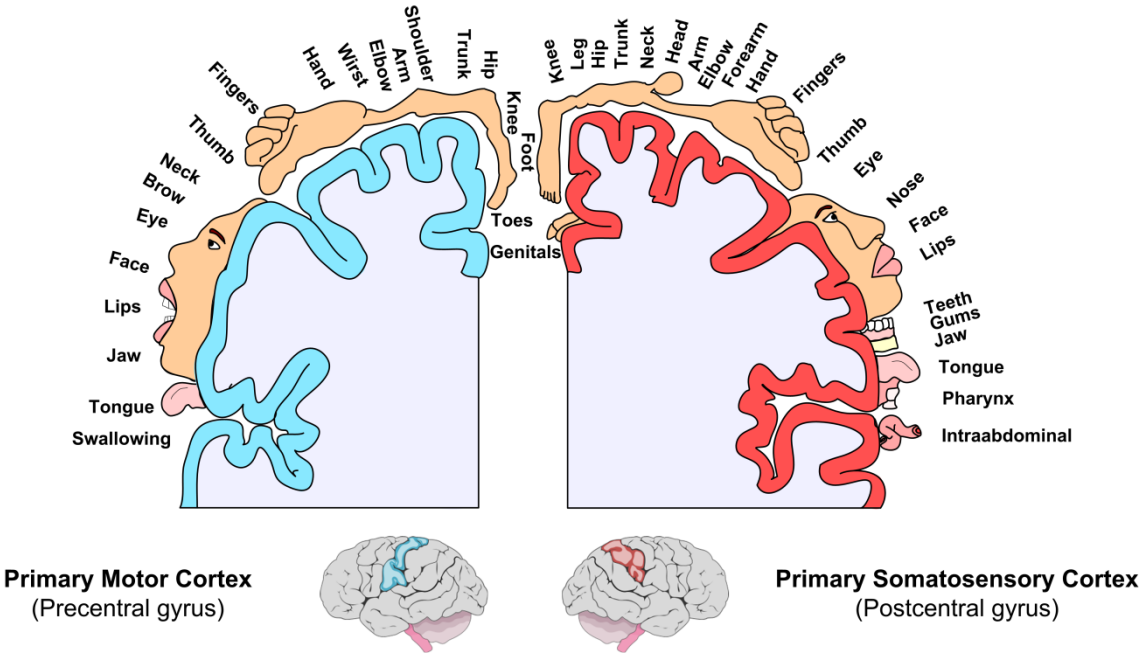

Figure 9: Representation of Penfield homunculus. Motor (in blue) and sensory (in red) strips are located in precentral and postcentral gyrus, respectively.

4.5. Efferent pathway

The localization of the motor neurons soma, which innervates the muscles involved in the deglutition process, depends on each specific nerve/cranial pair: facial and trigeminal are found in the brainstem bulge nucleus; ambiguus and hypoglossal nucleus are found in the

spinal bulb; and C1-C2 in the spinal cord. The axons of these neurons are projected through the cranial nerves (CN) V, VII, IX, X, XI and XII and the high-cervical nerves C1, C2 and C3 (that form the cervical loop).

Basically, the encephalic centres send impulses by CN motor neurons to all the effector muscles involved in swallowing:

- Oral phase and mastication: the facial muscles are innervated by CN VII; the chewing muscles by CN V; and the tongue by hypoglossal or CN XII.
- Swallow response: the suprahyoid muscles are innervated by CN V, VII and XII; the infrahyoid muscles by the cervical loop; the palate, pharynx and larynx by the pharyngeal branch of CN X, the branch of CN IX and X and superior cervical ganglia.

Finally, the larynx intrinsic muscles are innervated by motor neurons that have the soma located in the ambiguus nucleus and its axons are projected through the inferior laryngeal branch of CN X [17].

5. Normal swallow response from a biomechanical and neurophysiological perspective

In terms of biomechanics, the total duration of swallowing in healthy people is between 600 and 1000 ms, and includes fast reaction time of submentalis muscles [84], a short OSR (<740 ms) with fast times to LVC (<160 ms, measured from the opening of the GPJ to the closure of the LV) and to UESO (<220 ms, measured from the opening of the GPJ to the opening of the UES), high bolus velocity (>35 cm/s) and an intense lingual propulsion force (>0.33 mJ) (Figure 10) [85].

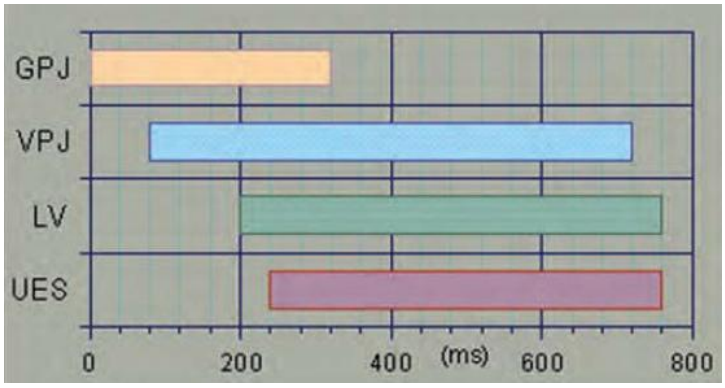

Figure 10: Example of a normal oropharyngeal swallow response chronogram. GPJ: glossopalatal junction; VPJ: velopharyngeal junction; LV: laryngeal vestibule; UES: upper esophageal sphincter.

Regarding the neurophysiological response, most of the studies were performed using pharyngeal sensory evoked potentials (pSEPs) to intrapharyngeal electrical stimulation that are characterized by having four peaks: the early peaks (N1 and P1) represent the conduction of the sensory stimuli from the pharynx to the brain cortex, while the late peaks (N2 and P2) represent the integration of the stimuli in the brain. In healthy young people the latency from the stimulus to each peak is about 80 ms for N1, 140 ms for P1, 190 ms for N2, and 300 ms for P2. Regarding the amplitudes, N1-P1, P1-N2 and N2-P2 are about 6 µV, 3 µV and 10 µV, respectively. In addition, deglutition sensory cortex representation is symmetric and bilateral (Figure 11) [82], [86]. In contrast, motor cortex representation is asymmetrical and bilateral, which means that the voluntary motor control of swallowing depends on hemispheric dominance, which is independent of handedness and unique for each person (Figure 12) [25].

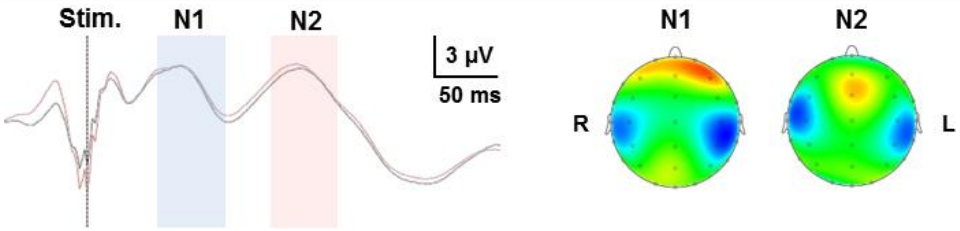

Figure 11: Representation of a pharyngeal sensory evoked potential and the scalp current density map of a healthy person, which are symmetric in both cases. Reproduced from Cabib et al (2017) [82].

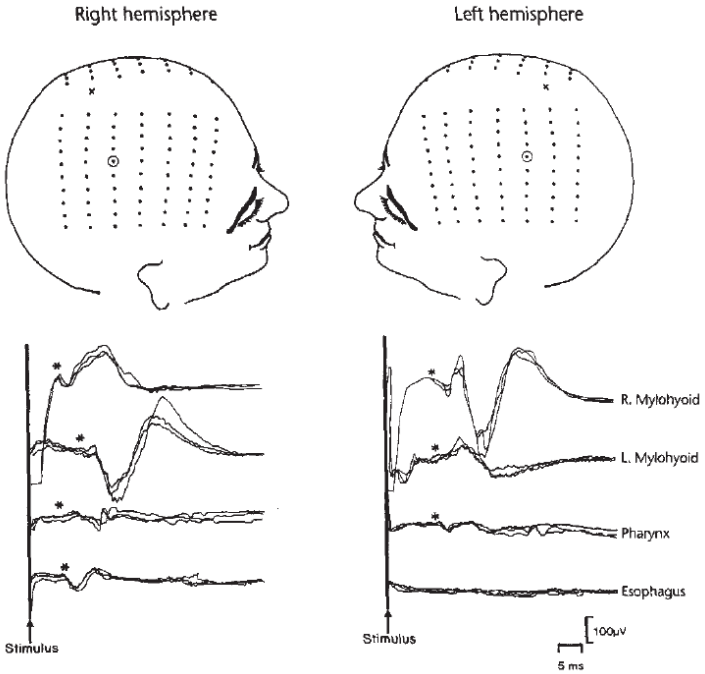

Figure 12: Example of myeloid muscle, pharyngeal and esophageal motor evoked potentials. Reproduced from Hamdy et al (1996) [25].

6. Oropharyngeal dysphagia (OD)

6.1. Prevalence

The prevalence of OD is very high, but difficult to determine exactly as it depends on the patient phenotype, its origin and the diagnostic method used, as shown in Table 1 [2]. The prevalence of OD in the main patient phenotypes included in this doctoral thesis are from 23 to 52% in older patients, from 57 to 84% in patients with neurodegenerative disease and from 40 to 81% in post-stroke patients. It is important to note that OD is infradiagnosed and the real prevalence will probably be higher, possibly similar to that of diabetes but less well known (Table 1).

Table 1: Prevalence of oropharyngeal dysphagia in several phenotypes of patients. Reproduced and modified from Clavé et al (2015) [2]. V-VST: Volume-Viscosity Swallow Test.

Phenotype	Target population	Evaluation method	Prevalence (%)	Reference
Older people	Independently-living	Screening (questionnaires)	11-38	Holland et al. 2011 [87] Roy et al. 2007 [88] Bloem et al. 1990 [89] Kawashima et al. 2004 [90] Yang et al. 2013 [91]
		Clinical exploration (V-VST)	23	Serra-Prat et al. 2011 [92]
	Hospitalized in an acute geriatric unit	Not specified/Clinical exploration (water swallow test or V-VST)	29.4–47.0	Lee et al. 1999 [93] Cabré et al. 2014 [94]
	Hospitalized with community-acquired pneumonia	Clinical exploration (water swallow test or V-VST)	55.0–91.7	Cabré et al. 2010 [95] Almirall et al. 2012 [96]
		Instrumental exploration	75	Almirall et al. 2012 [96]
	Institutionalized	Screening (questionnaires)	40	Nogueira & Reis 2013 [97]

		Clinical exploration (water swallow test)	38	
		Screening and clinical exploration	51	Lin et al. 2002 [98]
Stroke	Acute phase	Screening (questionnaires)	37-45	Martino et al. 2005 [99]
		Clinical exploration	51-55	
		Instrumental exploration	64-78	
	Chronic phase	Clinical exploration	25-45	
		Instrumental exploration	40-81	
Neurodegenerative disease	Parkinson's disease	Reported by patients	35	Kalf et al 2012 [100]
		Instrumental exploration	82	
	Alzheimer's disease	Instrumental exploration	57-84	Langmore et al. 2007 [101] Horner et al. 1994 [102]
	Dementia	Reported by patients	19-30	Langmore et al. 2007 [101] Ikeda et al. 2002 [103]
		Instrumental exploration	57-84	Suh et al. 2009 [104] Langmore et al. 2007 [101] Horner et al. 1994 [102]
	Multiple Sclerosis	Screening (questionnaires)	24	De Pauw et al. 2002 [105]
		Instrumental exploration	34.3	Calcagno et al. 2002 [106]
	Amyotrophic lateral sclerosis	Clinical and Instrumental exploration	47-86	Chen & Garrett 2005 [107] Ruoppolo et al.

				2013 [108]
Structural	Head and neck cancer	Clinical exploration	50.6	García-Peris 2007 [109]
		Instrumental exploration	38.5	Caudell et al. 2009 [110]
	Zenker's diverticulum	Instrumental exploration	86	Valenza et al. 2003 [111]
	Osteophytes	Screening	17-28	Utsinger et al. 1976 [112]

6.2. Causes and phenotypes

The main phenotypes of patients with OD are older patients, patients with neurological and neurodegenerative diseases, and patients following medical or surgical treatment for head and neck cancer. Recently OD has also been associated with COVID-19 and post COVID-19 patients [113]. In summary, OD has several causes: neurological, myopathic, structural, metabolic, infectious and iatrogenic (Table 2).

Table 2. Main causes of oropharyngeal dysphagia.

Oropharyngeal Dysphagia causes		
Neurological	**Myopathic**	**Structural**
Tumors of the brainstem	Connective tissue disease	Cricopharyngeal bar
Cranial trauma	Dermatomyositis	Zenker's diverticulum
Stroke	Myasthenia gravis	Cervical tissues (cervical webs)
Cerebral palsy	Myotonic dystrophy	Oropharyngeal tumors
Guillain-Barré	Oculopharyngeal dystrophy	Skeletal abnormalities/osteophytes
Huntington's disease	Polymyositis	Congenital structural abnormalities
Multiple sclerosis	Sarcoidosis	
Poliomyelitis	Paraneoplastic syndromes	
Post-polio syndrome		
Late dyskinesia		
Metabolic encephalopathy		
ALS		
Parkinson's disease		

Dementia		
Metabolic	**Infectious**	**Iatrogenic**
Amyloidosis Cushing's syndrome Thyrotoxicosis Wilson's disease	Diphtheria Botulism Lyme disease Syphilis Mucositis COVID-19	Medication side effects Post-surgical muscles Post-surgical neurogenics Radiation Corrosion Prolonged intubation

This doctoral thesis is mainly focused on patients with OD as a consequence of aging, stroke or neurodegenerative diseases.

6.2.1. Older patients

OD in older people seems to be caused by several processes related to aging, such as changes in head and neck anatomy and in muscular and neurological mechanisms, producing a functional impairment that could affect the swallowing mechanism. If this alteration in the deglutition response does not compromise the safety of swallowing then it is called presbyphagia. However, the characteristics of physiologically normal deglutition in healthy and robust older people are difficult to determine and it has not been established at what point the naturally slowing OSR can be considered dysphagia [114]–[117]. On the other hand, the many co-morbidities and polymedication in frail older people (who are defined as such if they have three of the following criteria: unintentional weight loss, exhaustion, weakness, slow walking speed, and low physical activity), suggest that this population is one of the most at risk of developing dysphagia [118]–[120]. In addition, OD meets the criteria to be considered a geriatric syndrome because it is very common in older patients, is related to multiple risk factors (aging, frailty, sarcopenia, neurogenic disorders, stroke, drugs that affect the oropharyngeal motor response, and head and neck cancer), and contributes to the development of various geriatric syndromes with poor prognosis and complications such as disability and frailty, functional impairment, malnutrition, hospital readmissions and morbidity and mortality [121].

In summary, older people have several factors (decreased cortical plasticity, olfaction, taste, dental status, muscle function, saliva secretion and tissue elasticity, and skeletal changes) associated with OD (Figure 13) [122].

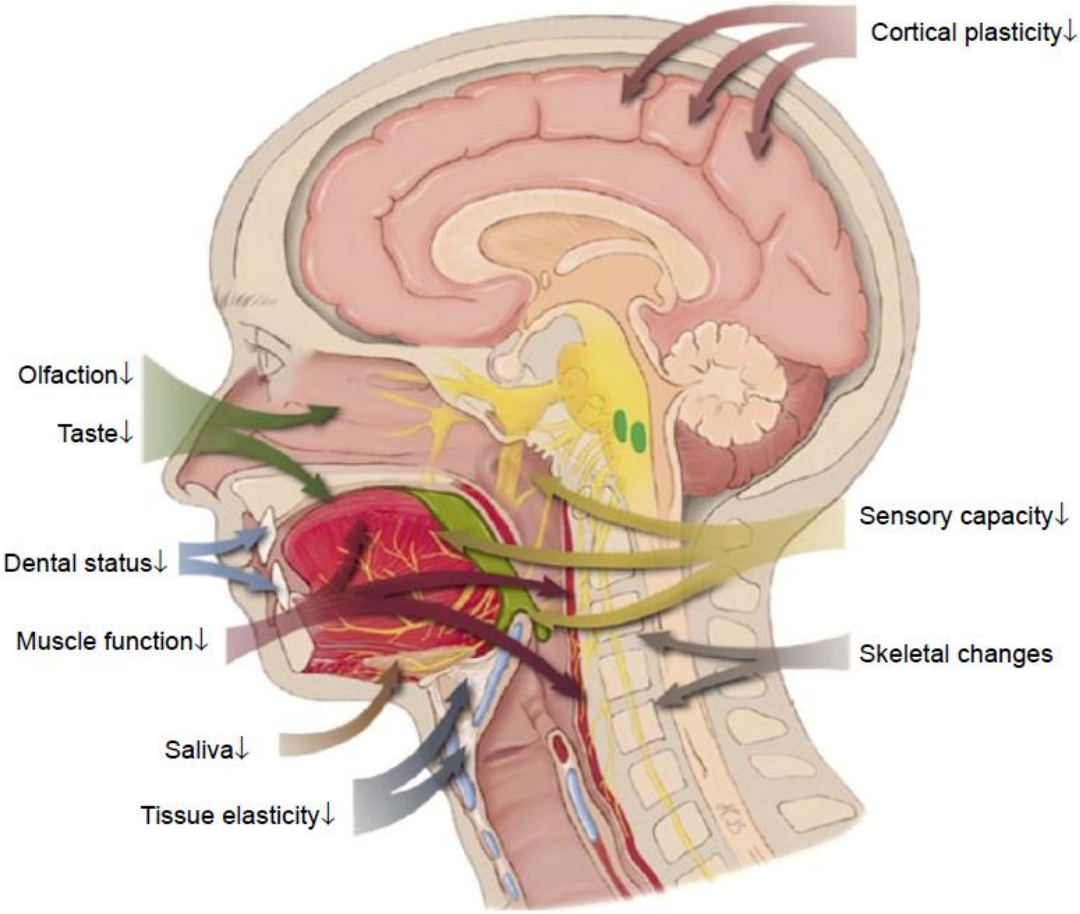

Figure 13: Factors associated with oropharyngeal dysphagia in older people. Reproduced from Wirth et al (2016) [122]. The arrow indicates decreased function.

6.2.2. Post-stroke patient

Because of the inter-hemispheric asymmetry of cortical activation during swallowing, unihemispheric stroke in the dominant hemisphere for swallowing is the main cause of developing OD in this phenotype of patient [25]. The prevalence of OD during admission has been reported at 40-45% and 41.7% at three months, and is an independent risk factor for prolonged hospitalization, institutionalization after discharge, and also for poorer functional capacity and increased mortality three month after stroke [123]. It is important to note that the impairments in deglutition revert in the first weeks after stroke in 42.4% of patients with unsafe swallows and in 30% of patients with ineffective swallows due to an increase in the pharyngeal motor representation in the contra-lesional hemisphere. In other patients, these

swallowing impairments persist, increasing the risk of nutritional and respiratory complications as a consequence of poor functionality [124]–[126].

6.2.3. Neurodegenerative disease patients

This phenotype of patients is the most heterogeneous due to the different diseases included in this classification (Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, etc.). The OD in these patients is due to both central and peripheral neurodegeneration processes as damage in the central and the peripheral sensory nerves has been observed when comparing patients with and without OD [127], [128]. The prevalence of OD in this phenotype of patients is very high independently of the disease: 82% in patients with Parkinson's disease; 85.9% in patients with dementia; and 80% in patients with multiple sclerosis. In all of them, more than 50% of patients with OD present safety impairments, causing aspiration pneumonia to be the main cause of death. In addition, OD in patients with neurodegenerative disease have a higher risk of poorer functionality and nutritional status, and also higher rates of respiratory infections and mortality at 18 months [129]–[132].

6.3. Pathophysiology

The common pathophysiology of OD in older and post-stroke patients and those with neurodegenerative disease is characterised by impairment in both biomechanics and the neurophysiology of swallowing.

6.3.1. Biomechanics

The impairment in biomechanics of swallowing in patients with OD is characterized by a delay in the initial deglutition phase (reconfiguration from the respiratory to the digestive pathway) which increases the duration of the OSR [20], [84], [85]. The most significant changes are the delay in the time to LVC, the main process that allows the protection of the respiratory tract during swallowing, and to UESO, causing an increased prevalence of penetrations of part of the bolus into the respiratory tract during the pharyngeal phase and the risk of regurgitation of the bolus by an increase in hypopharynx pressure, respectively [20], [85]. In addition, other common alterations observed in patients with OD is decreased lingual propulsion force, bolus velocity and kinetic energy, which increases the prevalence of oropharyngeal residue [85], [133]. These impairments in the kinematics, together with sarcopenia and increased time to UESO are responsible for oropharyngeal residue in older and neurogenic patients with OD [85], [119], [134].

OD in older patients is characterized by prolonged total duration of swallowing (1013±53 ms) and delay in time to LVC (476±48 ms) and to UESO (403±45 ms) [21], [85], [119]. Similarly, patients with post-stroke, dementia and Parkinson's disease and OD also showed an increased time to LVC (post-stroke patients: 416±129 ms; patients with dementia: 398±117 ms; patients with Parkinson's disease: 293±90 ms) but a preserved time to LV opening (LVO) and UESO [131], [133], [135].

As mentioned above, the LVC is the main process that protects the airway during deglutition, and a LVC cutoff of 340 ms in older and post-stroke patients and those with dementia [131]–[133] and 260 ms in patients with Parkinson's disease [135] predicts unsafe swallows in these phenotypes of patients.

6.3.2. Neurophysiology

The neurophysiological swallow response has been much less studied.

The aging process increases the latency and reduces the amplitude of the pSEPS peaks, more so in older people with OD in which the latency of N1 and N2 peaks increases to 80 ms and 250 ms, respectively, and the amplitudes of P2-N2 are reduced to 4 µV [86]. Similar results have also been found in patients with chronic post-stroke OD. In this case, patients showed an increased latency and reduced amplitude of pSEPs, but also a loss in the symmetry of the potentials and their cortical representation when comparing both brain hemispheres, the ipsilesional hemisphere presenting the reduced values [82]. In addition, the pharyngeal motor evoked potentials were symmetric in 73.3% of post-stroke patients with chronic OD without physiologic hemispheric dominance and with a significant reduction of cortical excitability of efferent pathways [136].

6.4. Clinical complications of OD

All the impairments of the OSR are translated into several signs and complications, which can be divided in two main groups:

- **Efficacy impairments**: these are characterized by the inability or difficulty of transporting the bolus due to the reduced tongue propulsion force and poor control of oropharyngeal muscles, causing the appearance of the most common sign of this group of disorders: oropharyngeal residue. Efficacy impairments can lead to malnutrition and dehydration as the patient cannot swallow sufficient liquids and nutrients. Both complications are related to low functional status and several

comorbidities such as sarcopenia, immunologic impairments and increased mortality [95], [137]–[142].

- **Safety impairments**: these are characterized by the entrance of part of the alimentary bolus into the respiratory tract as a consequence of the delayed time to LVC. Safety impairments can be differentiated into penetrations (part of the bolus enters into the LV without passing through the vocal cords) and aspirations (part of the bolus enters into the LV and passes below the vocal cords) and are classified according the Penetration-Aspiration Scale (PAS) [143]. When a patient with OD aspirates, pathogenic microorganisms that colonize the oral cavity and pharynx due to poor oral hygiene, and present in saliva, liquid or bolus, can reach the lungs causing a respiratory infection. This infection can lead to aspiration pneumonia, the first cause of death of these patients [2], [144], [145].

Considering each phenotype, in older patients with OD the main complications are high prevalence of malnutrition (45.3-51.1%), hypertonic dehydration, sarcopenia, reduced muscular and fat mass and weight loss. The worst complication in this patient phenotype is the development of respiratory complications, which become aspiration pneumonia in 10% of non-institutionalized people over 80 years old, and 10 times that number in institutionalized older and frail patients. The main risk factors of developing aspiration pneumonia in older people are poor dental hygiene, which is related to a change in oropharyngeal colonization, weak immune system, frailty and vulnerability and malnutrition [96], [140], [144], [146], [147]. In post-stroke patients, OD is associated with malnutrition, neurologic complications and respiratory and urinary infections, high mortality rates (Figure 14) and prolonged hospital stay. In addition, 30% of post-stroke patients develop aspiration pneumonia [123], [148], [149]. In patients with OD and neurodegenerative diseases, the most prevalent complications described are respiratory infections, malnutrition, dehydration and poor quality of life [132], [150].

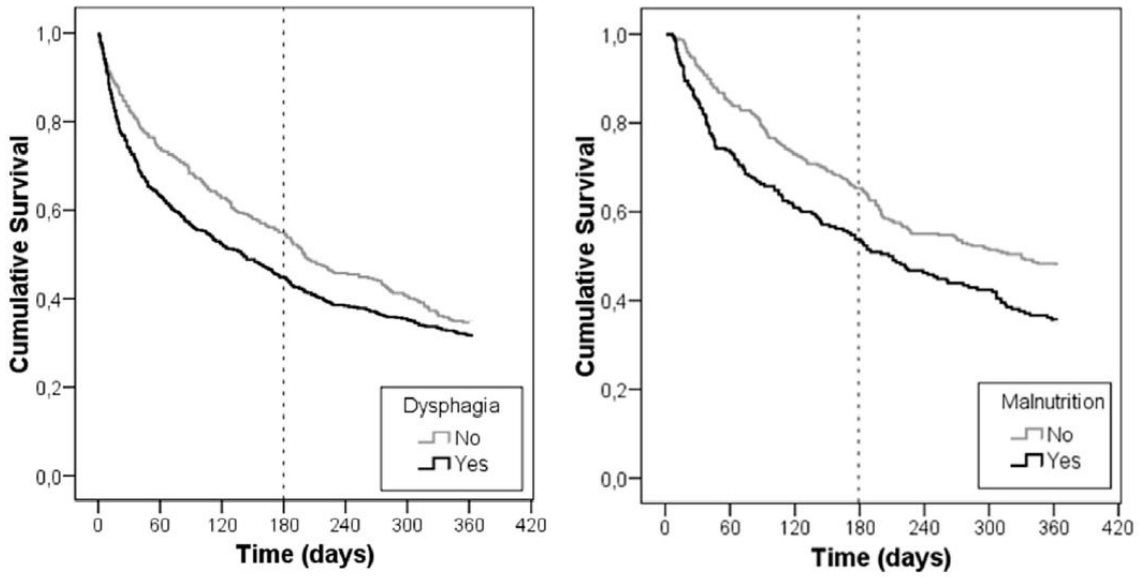

Figure 14: Cumulative survival curves for oropharyngeal dysphagia and malnutrition in older patients. Reproduced from Carrión et al (2014) [140].

6.5. Drugs related to OD

There are several drugs that are related to OD, which are prescribed for other disorders and have a beneficial or prejudicial effect on OD. The drugs that have a potential role of protecting against OD are beta blocking agents, selective calcium channel blockers, drugs acting on the renin-angiotensin system, non-steroid antiinflammatory and antirheumatic products and oral antidiabetics, as they seems to induce the swallowing reflex and protect against pneumonia [151]–[155]. On the other hand, the drugs with potentially harmful effects on the swallow response are antipsychotics, antidepressants and drugs for dementia[156]–[163], as patients who take these treatments double the risk of suffering OD [164].

6.6. Diagnosis

The diagnosis of OD needs to be made by a multidisciplinary team that identifies patients at risk using screening tools and consequently perform both clinical diagnosis tests (in order to identify clinical signs of impaired safety and efficacy of swallowand to make an early recommendation) and instrumental ones (with the aim of assessing the pathophysiology of OD in order to prescribe the most appropriate treatment).

6.6.1. Screening tools

Screening tools aim to detect patients at risk of unsafe swallows or aspirations [165]. These tools need to be easy and fast to perform so they can be applied systematically to screen all the patients at risk of OD. Two of the most commonly used specific tests are the Eating

Assessment tool-10 (EAT-10) [166], [167] and the Sydney Swallow Questionnaire [168]. If a patient shows risk of OD with any screening method, an exhaustive evaluation is needed using clinical and instrumental tools.

6.6.2. Clinical tools

Clinical tools aim to perform a first diagnostic, select those patients that need a more exhaustive evaluation and prescribe treatment to patients who cannot access instrumental assessment.

The clinical tools need to be performed by specialized personnel and one of the most complete is the **Volume-Viscosity Swallow Test (V-VST)**. This test helps to identify signs of both impaired efficacy (impaired labial seal, oral and pharyngeal residue and piecemeal deglutition) and safety (cough, voice change or wet voice and oxygen desaturation ≥3%), with a high sensitivity and specificity (93.17% and 81.39%, respectively) [169]. It is an effort test in which a series of boluses of different volumes and viscosities are administered in order of increasing difficulty. It starts with a bolus of 5ml of nectar viscosity (51-350 mPas·s) and continues with 10 and 20 ml boluses of the same viscosity. Then, the same algorithm is followed with liquid (<50 mPas·s) and pudding (>1750 mPas·s) viscosities. If the patient shows any sign of safety impairment during nectar and liquid series, the test will continue with the safest viscosity and volume (5ml pudding). In the case of a sign of impaired safety of swallow with any pudding bolus, the test will be interrupted [145], [170], [171].

6.6.3. Instrumental tools

Instrumental tools allow the clinician to confirm the diagnosis and to prescribe the best treatment plan. In addition they can be used to understand the pathophysiology of swallowing disorders and the effect of treatment on the swallow biomechanics. These tools evaluate the different swallowing structures and their function and also the role of volume and viscosity, postural changes and manoeuvres in respiratory tract protection [172].

6.6.3.1. Videofluoroscopy

Videofluoroscopy (VFS) is the reference diagnostic tool of swallowing disorders. It is a dynamic radiological exploration in which the patient is evaluated in a lateral projection while swallowing different boluses with radiological contrast. The image obtained includes the lips, oral cavity, pharynx, larynx, spine and oesophagus. The aims of the test are to assess the safety and efficacy of swallowing and to characterise the denominated VFS signs. The main VFS sign

of impaired efficacy is oropharyngeal residue (presence of part of the bolus in the mouth and/or the pharynx after swallowing) while the main signs of impaired safety are penetrations and aspirations. Subsequent image-by-image analysis of the VFS recording allows for qualitative (prevalence of safety and efficacy signs) and quantitative (timing of the OSR, kinematics of the bolus and hyoid and larynx movements) studies to be made of the swallow response [119], [133]. In our unit, the boluses administered to the patients during VFS consist of a 1:1 dilution of water and Omnipaque® (iodine radiopaque solution) and the required amount of thickening product to achieve 250 mPas·s and 800 mPas·s viscosities. These viscosities were selected in a previous study performed by our group which determined 250 and 800 mPas·s were the optimal viscosities to assess the swallowing function [173].

OD severity can be determined according to the degree of penetration or aspiration using VFS and the patient's reaction to these events (such as cough). This classification is performed using the **PAS** score [143], ranging from 1 to 8: 1 for normal deglutition, 2-5 for penetrations, and 6-8 for aspirations (Table 3). The scores 1 and 2 are interpreted as safe swallows (as a penetration degree of 2 is very slight and can be observed in healthy people) while the scores from 3 to 8 are considered unsafe swallows. In addition, residue can also be measured using the Robbins scale which evaluates the localization and the severity of oropharyngeal residue (Table 4) [174].

Table 3: Penetration-Aspiration scale score and its interpretation. Adapted from Rosenbek et al (1996) [143].

Score	**Description on VFS. Visuoperceptual sign.**
1	Material does not enter the airway.
2	Material enters the airway. Remains above vocal cords and is ejected from the airway.
3	Material is above vocal cords and is not ejected from the airway.
4	Material enters the airway, contacts vocal cords and is ejected from the airway.
5	Material contacts the vocal cords and is not ejected from the airway.
6	Material passes below the vocal cords and is ejected into larynx or out of the airway.
7	Material passes below the vocal cords and is not ejected from the trachea despite effort.
8	Material enters the airway, passes below the vocal cords and no effort is made to eject the material

Table 4: Description of Robbins scale for oropharyngeal residue.

Severity of residue on VFS	
Score	**Event**
0	No residue
1	Residue coating
2	Residue pooling
Locations	
Oral residue	Oral cavity
Pharyngeal residue	Vallecula
	Posterior pharyngeal wall
	Pyriform sinuses
	Upper esophageal sphincter

VFS also enables us to measure the timing of the OSR while swallowing a common bolus, which in the case of our group is the one of 5 ml nectar. Oropharyngeal reconfiguration is measured through the opening and closure times of the GPJ, the VPJ, the LV and UES. The time to GPJ opening is considered the time-point 0 (Figure 15) [119].

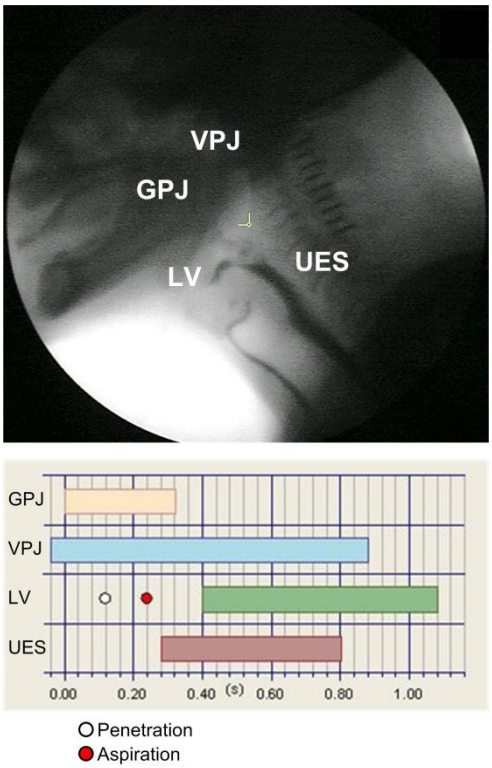

Figure 15: Example of a chronogram of the oropharyngeal swallow response in a patient with an aspiration. Note the delay in time to LVC associated with aspiration. GPJ: Glossopalatal junction; VPJ: Velopharyngeal junction; LV: laryngeal vestibule; UES: Upper esophageal sphincter.

In addition, the kinematics of the bolus can be determined: the mean bolus velocity is described as the time it takes from the entrance of the bolus through the GPJ to arrival at the UES divided by the distance between the GPJ and UES; the final bolus velocity is defined as the velocity of the bolus on arrival at the UES; and the tongue propulsion force is calculated using Newton's second law formula F=ma (m= bolus mass; a=bolus acceleration) and is expressed in mN [119].

6.6.3.2. Fiberoptic Endoscopic Evaluation of Swallowing

Fiberoptic endoscopic evaluation of swallowing (FEES) is an endoscopic tool used to assess swallowing and to visualize the pharynx and larynx structures and functions before, during and after swallowing. In addition, FEES reveals signs of efficiency impairments such as residue in the vallecula or the pyriform sinuses, and signs of safety impairments such as penetrations and aspirations. The equipment consists of a flexible fiberscope with light connected to a video device that records the sequence of images during the scan. It is a technique that is well tolerated, repeatable and can be performed at the patient's bedside. As with the VFS, several types of bolus (volumes and viscosities) can be used. The main limitation is that the oral phase cannot be visualized or evaluated [175]–[177]. However, FEES is a safe and well tolerated

technique and with low impact on the patient, even when performed by less experienced clinicians [178].

6.6.3.3. High Resolution Manometry (HRM)

Pharyngeal-esophageal high-resolution manometry (HRM) is used to assess and quantify the propulsive forces of the pharynx, the relaxation of the UES and the restrictive capacity of the UES through intravalvular pressure at the level of the hypopharynx. HRM uses a catheter that incorporates an array of solid state pressure sensors straddling the pharynx, UES and esophageal body. Pressure measurements are displayed using a contour color plot (Figure 16) and swallowing produces a velopharynx, pharyngeal stripping wave, UES movement/relaxation and proximal esophageal contraction. In recent years, efforts have been made to classify the different patterns observed by HRM [179]. HRM metrics define 3 relevant phenomena: 1) Tongue and pharyngeal propulsion, defined as the peak pharyngeal amplitude (mmHg), with ranges from 107 mmHg to 194 mmHg and influenced by age, volume and viscosity of the bolus and position of the person; 2) UES relaxation, the duration of which is the time between onset and offset of the UES pressure drop and the extent of which is defined as the minimum pressure during relaxation; and 3) pharyngeal intrabolus pressure, which is a marker of the resistance to bolus flow at UES level [180].

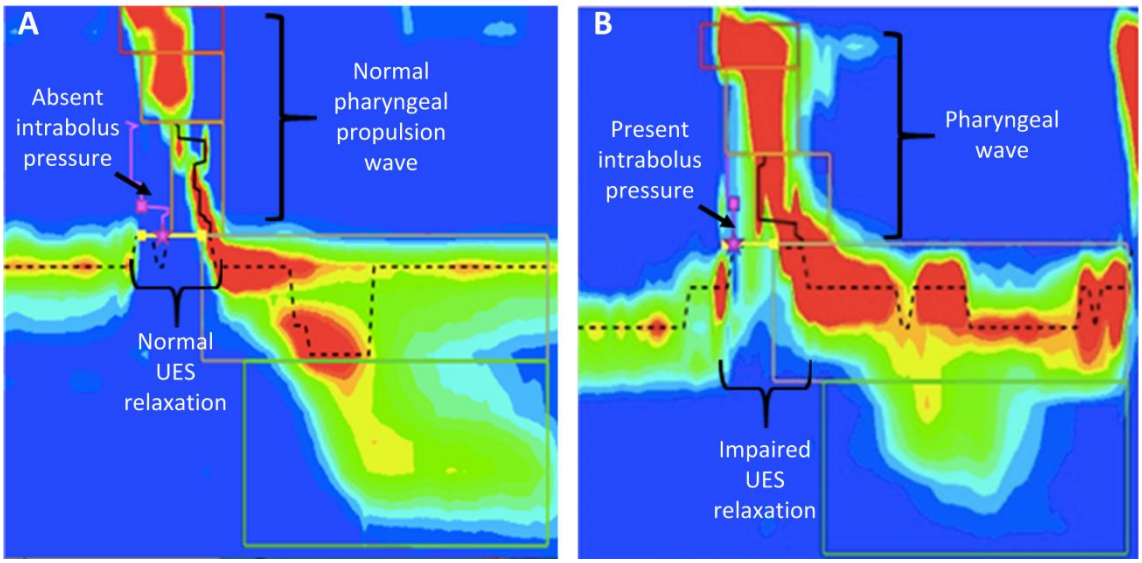

Figure 16: Examples of high-resolution manometry tracings of 5 ml swallow. Figure A shows a normal UES relaxation and a normal pharyngeal propulsion wave (Figure A). Figure B shows an incomplete UES relaxation that causes increased resistance to bolus leading to a high pharyngeal wave and increased intrabolus pressure at the hypopharynx level. UES: upper esophageal sphincter.

6.6.4. Neurophysiological tools

The afferent and the efferent pathways of the neurophysiological response of swallowing can be evaluated using pharyngeal sensory and motor evoked potentials.

6.6.4.1. Pharyngeal Sensory Evoked Potentials

Pharyngeal sensory evoked potentials (pSEPs) is the neurophysiological tool used to evaluate the afferent or sensory pathway. During an electroencephalogram (EEG), electrical stimuli are given to the pharynx with a nasopharyngeal probe, and the pharyngeal sensitivity and the latency and the amplitude of the characteristic peaks are measured (as explained in 5.3.2 section) (Figure 17). In addition, the EEG registers can be used to simulate brain activation by using the standardized brain electromagnetic tomography (sLORETA) software [181], [182].

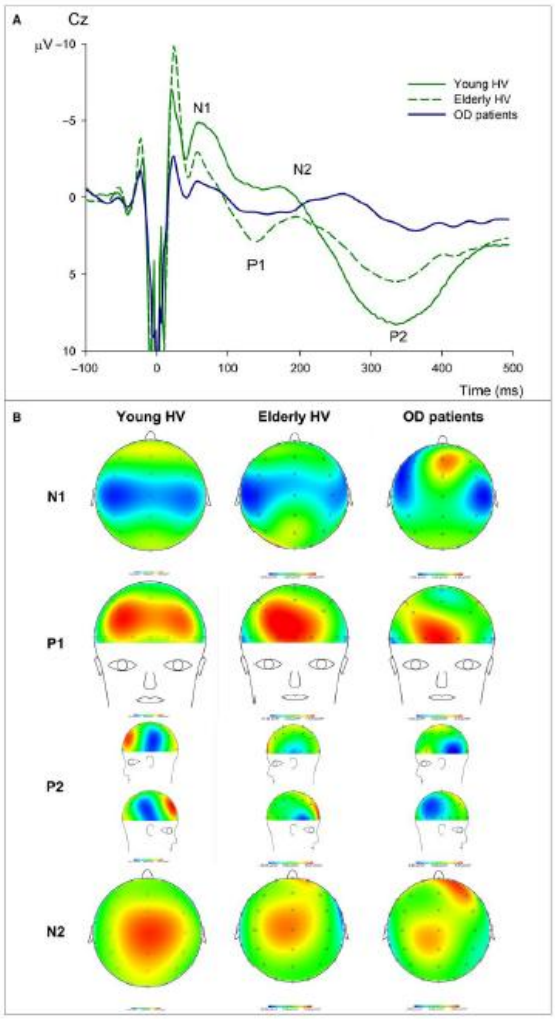

Figure 17: Example of (A) pharyngeal sensory evoked potential in young and older healthy volunteers (HV) and in older patients with oropharyngeal dysphagia (OD) and (B) the current scalp density maps. Reproduced from Rofes et al (2016) [86].

6.6.4.2. Pharyngeal Motor Evoked Potentials

Pharyngeal motor evoked potentials (pMEPs) is the neurophysiological tool used to assess the integrity of the efferent or motor pathway. It consists of stimulating the cortex areas that control pharyngeal movements using transcranial magnetic stimulation (TMS). The data is collected using a nasopharyngeal probe and the integrity of the motor pathway is measured through the amplitude, latency, duration and area under the curve of each potential.

7. Treatment. State of the Art

Current treatment of OD is based on compensatory strategies which avoid or reduce the signs of impaired efficacy and/or safety of swallow thus reducing the complications of OD.

7.1. Fluid thickening

Adaptation of fluid bolus viscosity is used to control and protect the respiratory airway [183]. Its main mechanism of action is based on reducing bolus velocity by increasing viscosity [184], this process significantly reduces the prevalence of signs of impaired safety of swallow but also increases the prevalence of oropharyngeal residue (Figure 18) [173], [185]–[188]. Changes in fluid bolus viscosity are achieved by adding thickening products to fluid, the final viscosity depends on multiple factors such as the type of thickening product (starch or gum), its intrinsic properties and the liquid basal characteristics (pH, temperature…) [183].

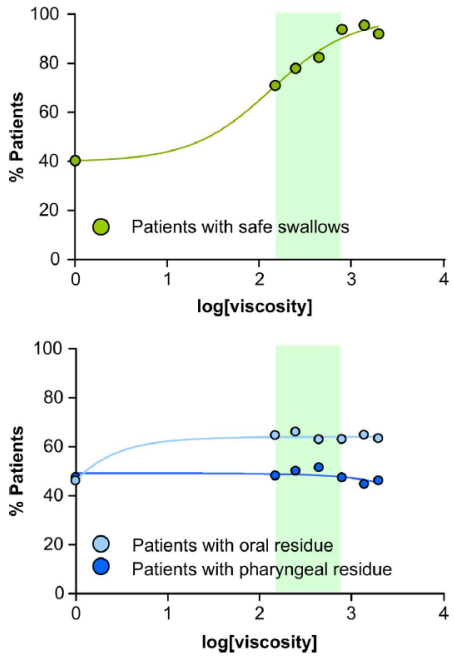

Figure 18: Example of dose-response curves for the therapeutic effect of a specific thickening product on safety and efficacy of swallowing. Reproduced from Bolívar-Prados et al (2019) [173].

Thickened fluids are classified as non-newtonian as their viscosity is affected by shear rate, which is defined as the rate at which a fluid is sheared during flow. The shear rate during swallowing is $50s^{-1}$ in the oral phase and $300s^{-1}$ in mesopharynx in patients with OD. Another factor that has been demonstrated to affect final viscosity is salivary alpha-amylase which breaks the O-glycoside bonds, very prevalent in starch based thickeners [189], [190]. These two factors affect shear viscosity, and, if not taken into account when making fluid adaptation recommendations, will endanger patients with OD [191].

There are several studies demonstrating the therapeutic effect of thickeners in reducing the prevalence of penetrations and aspirations, and their use has been correlated with reducing prevalence of respiratory infections, aspiration pneumonia and hospital readmissions [85], [119], [192]. However, treatment compliance is very low (48-56%), especially in patients that need higher viscosity levels, due to patients' dissatisfaction with taste and texture, difficulty swallowing, and discomfort preparing them [193].

7.2. Texture modified diets

Similar to fluid adaptation through the use of thickening products, texture modified diets aim to avoid aspirations and improve nutritional status and increase quality of life of patients with OD. Our group developed the triple adaptation, a nutritional plan based on the Mediterranean diet that is reproducible at home and meets three fundamental requirements: 1) rheological adaptation to ensure safe swallows; 2) nutritional adaptation to meet the necessary requirements according to the nutritional status of the patient; and 3) organoleptic adaptation in order to improve the taste, smell, presentation and palatability of the dishes offered to the patients [194].

7.3. Minimal massive intervention

The minimal massive intervention (MMI), developed in the Hospital de Mataró, consists of three interventions applied during hospitalization and discharge of patients with OD: 1) fluid thickening and texture-modified foods; 2) caloric and protein supplementation; and 3) oral health and hygiene. In contrast to standard clinical practice, the MMI improves nutritional status and functionality, reduces the prevalence of readmissions and respiratory infections, and increases survival at 6-month [195]. These results demonstrate how a simple and cost-effective clinical practice can avoid OD complications in older patients with OD.

7.4. Postural changes, manoeuvres and neuromuscular praxis

Postural strategies can modify the direction of the bolus direction and totally or partially reduce the prevalence of aspirations and oropharyngeal residue. Anterior neck flexion protects the airway while posterior flexion facilitates pharyngeal gravitational drainage and improves oral transit speed. Rotation of the head towards the paralyzed pharyngeal side directs the bolus towards the healthy side, increasing the efficiency of the pharyngeal transit and facilitating the opening of the UES. In contrast, the aim of manoeuvres is to biomechanically compensate physiological impairments in order to protect the respiratory tract. Manoeuvres are voluntary movements that require the ability of the patient to learn and perform them [89], [172], [196], [197]. The most common manoeuvres are the supraglottic swallowing, supersupraglottic swallowing, forced swallowing, Mendelsohn's manoeuvre and Masako's manoeuvre. Finally, neuromuscular praxis consists of repetitive exercises to train specific oropharyngeal or neck muscles in order to improve swallowing physiology, the best known being the Shaker's manoeuvre [21], [198]–[200].

8. Neurorehabilitation treatments

8.1. Peripheral neurostimulation

8.1.1. Mechanical and thermal stimulation

Mechanical and thermal stimuli have been used in the past to find the pharyngeal sensory areas that trigger deglutition and to measure the loss of pharyngeal sensitivity in OD patients and populations at risk of dysphagia [116], [201]–[203]. They have also been used in acute treatments of neurogenic patients with OD to improve swallowing physiology. For instance, cold and mechanical stimulation of the glossopharyngeal innervated regions significantly reduced total transit time in neurogenic patients with OD [204]. These results were reproduced using a metallic instrument that provided 0-3°C tactile and cold stimuli in the same area in patients with idiopathic Parkinson's disease and OD [205]. Mechanical stimuli with air pulses has been used as a treatment for stroke patients with OD, improving the swallowing frequency [206]. Mechanical thermal stimulation of the oropharynx improves the OSR of neurogenic OD patients by increasing the sensorimotor cortical activation [207].

8.1.2. Chemical stimulation

8.1.2.1. Acid

One of the first approaches using chemical sensory stimulation as a therapeutic strategy to treat OD was with sour boluses (50% v/v citric acid from lemon juice). This strategy reduced the prevalence of aspirations by shortening the pharyngeal swallow delay in patients with neurogenic OD but the high concentration of citric acid was not well tolerated by patients [208]. Later studies proved that an equivalent concentration of citric acid (2.7% w/v) achieved a similar therapeutic effect but mixing citric acid and sucrose (1.11% and 8% w/v respectively) did not significantly improve OD symptoms or swallow response parameters [209]. Finally, stimulation with both cold (2-8°C) and acid stimuli (citric acid from lemon juice) reduced the pharyngeal swallowing time in stroke patients [210], [211].

8.1.2.2. Carbonation

Several clinical studies have tested the use of carbonated fluids (obtained by mixing citric acid and sodium bicarbonate) in neurogenic patients with OD; analysing deglutition with VFS, these studies proved that carbonated fluids reduce the prevalence of laryngeal vestibule penetrations and pharyngeal residues by improving the OSR [212]–[214]. Studies on healthy volunteers have shown that stimulation with carbonated liquids increases corticobulbar excitability, which would explain the OSR improvement in patients with OD [215].

8.1.2.3. Pharmacologic stimulation of TRP agonists

Another early attempt to use chemical stimulation of the swallow response was with capsaicin (10^{-6}-10^{-9} M), the pungent substance found in red chilli peppers. In early studies, drops were directly inserted into the pharynx, and this reduced the swallow reflex latency in stroke and dementia patients in a dose-dependent manner [216]. Some years later, capsaicin and other pungent molecules such as piperine, the pungent substance found in black pepper, were used on patients with OD caused by either age, stroke or neurodegenerative diseases. In both acute and sub-acute clinical trials, capsaicin and capsaicinoids (in a concentration of 150μM or 10μM) were administered in the bolus to patients with OD and reduced prevalence of both safety and efficacy impairments by shortening the laryngeal vestibule closure time and increasing the bolus velocity [217]–[219]. In addition they have been shown to improve the efficacy of swallowing by increasing the pharyngeal contractile integral and the time to UESO activation and relaxation [220]. Moreover, it has been shown that the stimulation of TRPV1 with capsaicin induced the secretion of SP and CGRP, increasing its concentration in patients' saliva [220]–[222].

Olfactory stimulation with black pepper oil and oral stimulation with piperine within the bolus (10^{-4}-10^{-3} M) also significantly reduced prevalence of impaired safety of swallow in patients with OD [223], [224] and, when applied during a long period of time (30 days), it even enhanced neural plasticity in the left insular cortex [223]. Finally, the use of menthol, either inserted into the pharynx (10^{-4}-10^{-2} M) or mixed with the bolus (10^{-3}-10^{-2} M), reduced the prevalence of safety impairment by improving the swallow response [225], [226]. However, compared to the therapeutic effect of capsaicinoids or piperine, menthol is the least effective of these agonists [226]. A combination of all these treatments was used in patients with OD admitted to hospital with recurrent pneumonia prior to reintroducing oral feeding. Starting with three days of olfactory stimulation with black pepper oil once pneumonia had been cured, capsaicin troches were then administered for five days and, finally, a menthol flavoured jelly was fed to patients prior to oral feeding introduced in a step by step manner, reducing as a consequence the incidence of pneumonia [227]. Although acute clinical trials have been done as proof of concept to assess the therapeutic effect of these chemical and pharmacological strategies, more mid and long term studies will be needed to assess the long term effect of a chronic treatment using these strategies. Long term studies are also needed to confirm the effect of these strategies on the CNS.

8.1.2.4. Electrical stimulation

The swallow response can be elicited by direct electrical stimulation of the Ph-IX nerve and the ISLN. However, electrical stimulation of the L-IX nerve inhibits swallow and electrical stimulation of the Ph-X nerve has no effect on the swallow response [42]. Dependent-voltage channels of the sensory neurons of these nerves might be activated by sensory electrical stimulation and thus the input signal conducted to superior areas of the CNS.

Electrical stimulation therapies are currently being performed in two modalities:

- Intrapharyngeal electrical stimulation applies electrical stimuli on the pharynx using intrapharyngeal electrodes. The application of this treatment, either acutely (for 10 min) or subacutely (several days of treatment) in contrast to sham, in post-stroke patients with OD, produces a significant improvement in the swallow response by reducing the pharyngeal transit time, swallowing response time and prevalence of aspirations [228]–[231]. In addition, it reduces hospital length of stay of these patients [232] and increases the concentration of SP in saliva [233].
- Transcutaneous electrical stimulation is used to activate the muscles involved in the swallowing function through the peripheral motor nerves (neuromuscular electrical

stimulation) [234]–[238]. Another treatment strategy is sensory stimulation, using lower electrical intensity to avoid muscle contraction during treatment and stimulate sensory nerves. This approach has shown significant improvements in several swallow parameters, such as reduced swallow response time and prevalence of aspirations in chronic post-stroke dysphagic patients [238]–[240].

8.2. Central stimulation. Non Invasive Brain Stimulation Techniques

The objective of central stimulation is to induce neuroplasticity through the direct stimulation of the brain cortex. There are two main techniques denominated non-invasive brain stimulation: repetitive transcranial magnetic stimulation and transcranial direct current stimulation. Little is known about their therapeutic effect but both strategies seem to have a positive effect on patients with post-stroke OD by improving the OSR [241]–[245].

HYPOTHESIS AND OBJECTIVES

1. Hypothesis

H1. Acute stimulation of TRP receptors located in sensory nerves of the human oropharynx, with TRPV1, TRPA1 or TRPM8 agonists added to the alimentary bolus, stimulates both the central pattern generator in the brainstem and the pharyngeal sensory cortex, inducing a significant improvement in both the biomechanics and neurophysiology of swallowing in older patients with oropharyngeal dysphagia.

H2. Compensatory treatments with thickening agents improve safety of swallowing in patients with oropharyngeal dysphagia without major changes in the swallow response. In contrast, acute treatment with TRP agonists causes both significant improvements in the safety of swallow and in the biomechanics and neurophysiology of swallowing.

H3. Sub-acute treatment with a low dose of TRP agonists improves the biomechanics and neurophysiology of swallowing without inducing desensitization.

H4. The basal salivary concentration of the neuropeptides substance P and calcitonin gene-related peptide could be a good peripheral and non-invasive biomarker of pharyngeal sensory function.

2. Objectives

O1. To determine the acute therapeutic effect of oropharyngeal stimulation with TRP agonists on the biomechanics and neurophysiology of swallowing in patients with oropharyngeal dysphagia. To assess the acute effect of TRP agonists on voluntary swallows and involuntary spontaneous swallowing frequency.

O2. To compare, with videofluoroscopy and neurophysiological explorations, the acute therapeutic effect of active treatments based on TRP agonists with those based on starch- and gum-based thickening products.

O3. To determine the sub-acute therapeutic effect of oral TRPV1 agonists before meals over 10 days on the biomechanics and neurophysiology of swallowing in patients with oropharyngeal dysphagia.

O4. To evaluate the basal concentration of the neuropeptides substance P and calcitonin gene-related peptide in saliva in young healthy volunteers, older people without oropharyngeal dysphagia and older patients with oropharyngeal dysphagia and to correlate it with pharyngeal sensitivity assessed by electrical stimulus.

PATIENTS, MATERIAL AND METHODS

1. Ethical statement

All the studies in this doctoral thesis that were performed with patients and healthy volunteers included in Chapters 1, 3 and 4 were reviewed and approved by the Ethical Committee of the Hospital de Mataró with the following codes: Chapter 1.1: CEIm code 55/14 ; Chapter 1.2: CEIm code 11/17; Chapter 3: CEIm code 04/12; and Chapter 4: CEIm code 3/17. In addition, the clinical studies are registered in ClinicalTrials.gov: NCT02422576 (Chapter 1.1) NCT01762228 (Chapter 3).

All the procedures were conducted according to good clinical practices (GCP) and to the rules of the Declaration of Helsinki and its last amendments and all the participants signed informed consent.

This doctoral thesis is composed of four chapters as detailed below. The methodological part of the thesis will be described in sections and include descriptions of each chapter: Patients; Investigational products; Biomechanical evaluation; Neurophysiological evaluation; Saliva sample collection. SP and CGRP quantification; and Statistical analysis.

- Chapter 1: Evaluation of the acute therapeutic effect of TRP agonists
- Chapter 2: Comparative study of the acute therapeutic effect of TRP agonists
- Chapter 3: Evaluation of sub-acute therapeutic effect of TRPV1 agonist
- Chapter 4: Description of a peripheral biomarker of pharyngeal sensitivity

2. Patients

The population of each of the studies included in the doctoral thesis is detailed below:

Chapter 1.1:

191 patients were screened with the V-VST and those who fulfilled the inclusion criteria (58 patients) were randomized into one of the three treatment groups: 21 patients in the cinnamaldehyde-zinc (CIN-Zn) group, 21 patients in the citral (CIT) group, and 16 in the citral-isopulegol group (CIT-ISO). The inclusion criteria were to be older than 55 years, have signs of impaired safety of swallow during the V-VST, and that the cause of OD was neurodegenerative disease, stroke (>3 months), or aging.

Chapter 1.2:

A total of 141 healthy volunteers (18–90 years old) were recruited from the community and divided into three groups according to age: 50 in the 18–39 age group (GI), 49 in the 40–59 age group (GII), and 42 in the >60 age group (GIII). The inclusion criteria were to be moret han 18 years old without previous diagnosis of swallowing disorders or using any medication that could influence the saliva flow (such as antidepressants, antipsychotics, or anticholinergics). The exclusion criteria were head and neck surgery/radiotherapy or neurological disorders including neurodegenerative diseases. In addition, 17 acute stroke patients were recruited through the Neurology Department of the Hospital de Mataró. All patients had signs of impaired safety of swallow according to the V-VST. The exclusion criteria were to have a life expectancy of less than three months, OD diagnosis previous to the stroke episode, or OD associated with pathology other than stroke.

Chapter 2:

Data from 329 patients with OD as a consequence of aging, stroke or neurodegenerative disease from six studies [185], [218], [224], [226], [246], [247] were collected, of whom 148 received an active treatment based on TRP agonists (33 patients were treated with capsaicin 150 µM; 7 patients with capsaicin 10 µM; 20 patients with piperine 1mM; 20 patients with piperine 150 µM; 20 patients with menthol 10 mM, 20 with menthol 1mM; 21 with cinnamaldehyde-zinc 200 ppm-70 µM; 21 with citral 200 ppm; and 16 with citral-isopulegol 200-250 ppm) and 151 with the usual compensatory treatment based on thickening products (33 received a modified starch based thickener; and 118 a xanthan gum based thickener).

Chapter 3:

A total of 28 older patients with OD associated with aging were included in the study: 14 in the acute or single-dose study, and 14 in the sub-acute or multiple dose study. In both studies, patients were randomized in the placebo or capsaicinoids intervention group. Inclusion criteria were for patients to be more than 70 years old, in a stable medical condition and to have clinical signs of OD according to the V-VST.

Chapter 4:

A total of 43 participants were included in the study: 15 young (18–55 years) healthy volunteers, 14 older (>65 years) people without OD and 14 older people with OD. Inclusion criteria were to be aged between 18 and 50 years for the healthy volunteers and more than 65 years for the healthy elderly and elderly with OD, in a stable medical condition, without history

of neurologic or neurodegenerative diseases for the healthy elderly and to have impaired safety of swallow (PAS>2) according to VFS for the elderly with OD.

3. Investigational products

The investigational products (IP) tested in this doctoral thesis, all of them offered to the patients by adding them into a nectar viscosity bolus, were:

Chapter 1.1:

The patients received one of the following IP during the VFS (two nectar series of 5, 10 and 20 ml supplemented with one of the IP) and pSEPs recording (two administrations of 35 ml of supplemented nectar with the same IP they received during the VFS) according to the group they were randomized into:

1. The TRPA1 agonist Cinnamaldehyde (756.6μM/100ppm) and Zinc (70μM) [248].
2. The TRPA1 agonist Citral (1.6mM/250ppm).
3. The combined TRPA1-TRPM8 agonist Citral (1.6mM/250ppm) and Isopulegol (1.3mM/200ppm).

All these IPs were prepared by Nestec Ltd.

Chapter 1.2:

All post-stroke patients with OD were given four 5 ml boluses of TRPV1 agonist capsaicin (Spectrum chemical MFG Corp, New Brunswick, NJ, USA) in a concentration of 10μM. The solution was prepared by the Pharmacy Department of the Universitat de Barcelona.

Chapter 2:

All TRP agonists studied by our group were included in this retrospective study. In addition to the agonists described in Chapters 1.1 (Cinnamaldehyde-Zinc, Citral and Citral-Isopulegol) and 3 (Capsaicinoids), the following TRP agonists were included:

- TRPV1 agonist: Capsaicin 150μM [218].
- TRPA1/TRPV1 agonist: Piperine 1mM and 150μM [224].
- TRPM8 agonist: Menthol 1mM and 10mM [226].

Details of how each TRP agonist was prepared are explained in each of the referenced studies.

Chapter 3:

For this study the supplementation of the nectar boluses was prepared by our team by adding Tabasco sauce. Capsaicin (TRPV1 agonist) concentration in the capsaicinoids sauce (McIlhenny Co, Avery Island, LA, USA) was 185.5 μg/g, measured with liquid chromatography (AOAC 995.03 method).

In the single-dose protocol, participants were given 10 ml of a nectar solution of capsaicinoids 10μM (active group) or placebo (potassium sorbate) (control group). In the multiple-dose protocol, participants were given 10 ml of a nectar-like solution of capsaicinoids 10μM or placebo three times/day (before meals) for 2 weeks (5 days/ week).

4. Biomechanical evaluation

4.1. V-VST

The aim of the V-VST assessment is to identify clinical signs of impaired efficacy of swallow, such as impaired labial seal, oral or pharyngeal residue, piecemeal deglutition (multiple swallows per bolus), and clinical signs of impaired safety during swallow such as changes in voice quality (including wet voice), cough or a decrease in oxygen saturation ≥3% measured with a finger pulse-oximeter. The probe of the pulse-oximeter is placed on the index finger of the right hand and baseline readings are obtained 2 min prior to starting the test. Cough, voice changes and/or fall in oxygen saturation ≥3% are considered major clinical signs of safety impairments of swallowing. The V-VST is designed to protect patients from aspiration by starting with nectar viscosity and increasing volumes from 5 mL, to 10 mL and 20 mL boluses in a progression of increasing difficulty. When patients have completed the nectar series without major symptoms of aspiration (cough, voice changes and/or fall in oxygen saturation ≥3%), a liquid viscosity series will be assessed also with boluses of increasing difficulty (5 mL to 20 mL). Finally, a safer pudding viscosity series (5 mL to 20 mL) will be assessed in the same way. If the patient presents signs of impaired safety at nectar viscosity, the series will be interrupted, the liquid series will be omitted, and a safer pudding viscosity series will be assessed. If the patient presents signs of impaired safety at liquid viscosity, the liquid series will be interrupted, and the pudding series will be assessed (Figure 1).

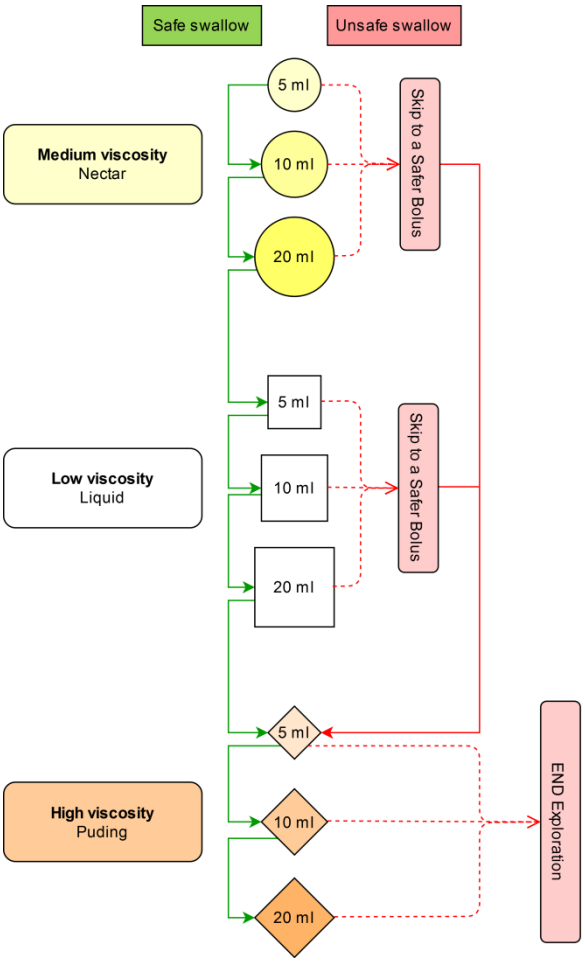

Figure 1: Volume-viscosity swallow test algorithm. Reproduced from Riera et al (2021) [169].

4.2. VFS

VFS was performed on all patients, seated in a lateral projection, and included the oral cavity, the pharynx, the larynx and the cervical esophagus. The VFS was obtained with the x-ray equipment Super XT-20 Toshiba Intensifier (Toshiba Medical Systems Europe, Zoetermeer, The Netherlands) and recorded at 25 frames/second for later analysis with a Canon 3CCD digital video camrecorder XM2 (Canon Inc, Tokyo, Japan). The VFS algorithmis were the same as for the V-VST, following the same order of administration of the different viscosities and volumes and ensuring the safety rule. However, VFS allows the severity of the signs of impaired efficacy and safety of swallowing to be quantified, as well as measuring the times of the OSR as it is an x-ray assessment and the bolus administered to the patient had iodine radiopaque solution. Signs of impaired efficacy of swallow include labial seal, piecemeal deglutition and oral, pyriform sinus and vallecula residue. The safety of swallowing was measured with the PAS score, as explained before, considering that a score higher than 2 indicates safety impairment

(Figure 2). The main OSR parameters measured were the time to LVC (protection of the airway), to UESO and to LVO (total duration of swallow).

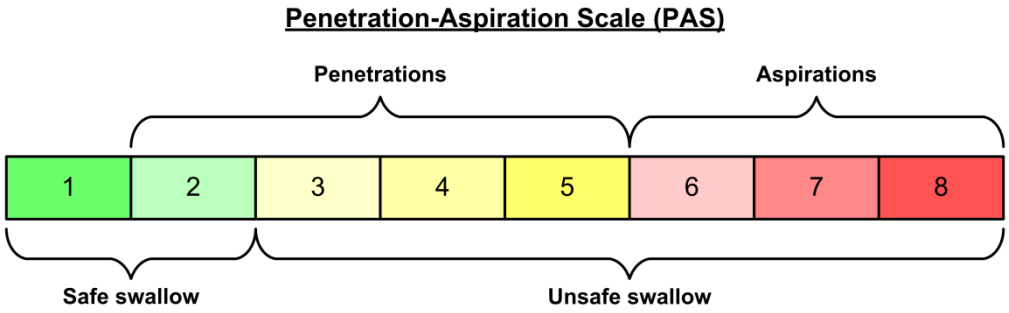

Figure 2: Representation of penetration-aspiration scale (PAS).

VFS signs of impaired safety and efficacy of swallow and swallow biomechanics were analyzed offline with the software Swallowing Observer (Image and Physiology SL, Barcelona, Spain).

In addition, the VFS examination included in Chapter 1.1 and 3 were modified to adapt them to the aim of these studies. The variations in the VFS protocols are:

Chapter 1.1:

During VFS, the patient took three nectar series: a control series (T0) with 5, 10, and 20 mL boluses of nectar viscosity (181.92 ± 1.75 mPa·s at 50 s^{-1}); after 2 minutes, another series of 5, 10, and 20 mL nectar viscosity boluses with the IP (T1); and after 5 minutes, another series of nectar viscosity boluses containing the IP (T2).

Chapter 3:

In the single-dose protocol patients were studied during the deglutition of one series of 5 ml, 10 ml and 20 ml nectar-viscosity boluses (274.42±13.14 mPas·s) as a control series, followed by a sensitization of two 5 ml boluses supplemented with capsaicinoids (10μM) 2 minutes apart. Finally, 5 minutes after the sensitization, two more series of 5 ml, 10 ml and 20 ml nectar boluses supplemented with capsaicinoids (10μM) were administered.

4.3. Spontaneous swallowing frequency (SSF)

Spontaneous swallowing frequency (SSF) was measured in Chapter 1.2 with surface neck electromyography (EMG) and accelerometry. After cleaning the skin with alcohol, EMG electrodes were positioned over the suprahyoid muscles, while an omnidirectional accelerometer was placed over the cricothyroid cartilage. This omnidirectional accelerometer assesses acceleration in multiple directions but is more sensitive to movement in the vertical plane. SSF was recorded for 10 minutes. All recordings were analyzed offline using the AcqKnowledge software (BIOPAC Systems Inc., California, USA), which displays a visual trace of

the EMG, the complete EMG and the accelerometer signal. A spontaneous swallow was considered when the signal was registered by both EMG and the accelerometer. The spontaneous swallowing rate was calculated as spontaneous swallows per minute. In addition, the following EMG metrics were analyzed for each swallow: amplitude (distance from highest to lowest peak of the EMG signal expressed in volts), duration, delta T (ΔT, time from start to end in seconds) and area under the curve (AUC, amplitude and ΔT integral, in volts·seconds).

5. Neurophysiological evaluation

5.1. Pharyngeal sensory and tolerance threshold

The pharyngeal sensory and tolerance thresholds to electrical stimulation were determined for each participant in Chapters 1.1, 3 and 4. The electrical stimuli were applied through an intrapharyngeal catheter with two bipolar electrodes (Gaeltec Ltd, Dunvegan, Scotland) that were positioned at 14-15 cm from the nostril. The catheter was connected to a Digitimer DS7A current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom). Both thresholds were determined by triplicate, applying several stimuli of 0.2 ms at increasing intensity in steps of 0.5 mA. The sensory threshold was defined as the electrical stimulus intensity that patients' first perceive, while the tolerance threshold corresponded to the highest intensity that they felt comfortable with.

5.2. Pharyngeal sensory evoked potentials

Patients were studied by electroencephalography (EEG) during the application of several trains of electrical stimuli to their pharynx delivered with the same intrapharyngeal catheter described above. The cortical response to electrical stimuli was recorded with a 32 scalp tin electrodes cap (ElectroCap Internacional Inc, Eaton OH, USA) an amplification of the 10–20 system, referenced to the left ear lobe and connected to a BrainAmp amplifier (Brain Products GmbH, Gilching, Germany). The ground electrode was included in the EEG cap and located just below the fronto-central electrode FCz. An electrode was placed on the skin below the left eye to record the vertical electrooculogram. Electrode gel was applied to each electrode to keep impedance below 5 KΩ. The signal was digitized at a sampling rate of 500 Hz and filtered with a 50 Hz notch. Recordings were performed in a quiet room with the patient seated, awake and with eyes open. The patient was asked to stay calm and relaxed. EEG activity was recorded while the patient received several stimuli sets. These sets of electrical stimuli lasted 4:15 min, with an inter-set interval of 1 min, and consisted of 100 square wave 0.2 ms with an inter-stimuli interval of five seconds at 75% intensity of the tolerance threshold.

Once registered, the pSEPs were analyzed offline and processed with BrainVision Analyzer Software 2.0 (Brain Products), filtered, and corrected for eye blink. Based on the results of the pSEPs analysis, the time frames used to compute the cortical activity distribution were 56-80ms for the N1 peak, 120-150ms for the P1 peak, 220-270ms for the N2 peak and 300-350ms for the P2 peak. Finally, pSEPs of each group of patients were averaged to obtain the grand average.

The number of sets of stimuli used to assess pSEPs differs depending on the protocol:

Chapter 1.1:

Three sets were used in total: 1 before the treatment (T0) and 2 more after the administration of 35ml nectar supplemented with the IP or placebo (T1 and T2).

Chapter 3:

In both protocols the patient received 4 sets of stimuli:

- In the acute protocol, after the two first sets were administered, 10 ml of nectar supplemented with capsaicinoids or placebo were given followed by two more sets of stimuli.
- In the sub-acute protocol, the four sets were registered with the inter-sets interval previously described before and after the 10-day treatment with capsaicinoids.

5.3. Neurotopography

The brain source of each pSEP component was localized using the standardized brain electromagnetic tomography (sLORETA) software [181], [182]. Computations were made on a head model using the Montreal Neurological Institute 152 template with the three-dimensional solution space restricted to cortical gray matter, as determined by the probabilistic Talairach atlas. The intracerebral volume is partitioned in 6239 voxels at 5mm spatial resolution. Anatomical labels like Brodmann areas (BA) are also reported using standard stereotactic space, with correction to Talairach space.

6. Saliva sample collection. SP and CGRP quantification.

Saliva samples analyzed in Chapter 4 were gathered at the same time of day (from 8 am to 12 pm) from all participants in order to avoid the circadian rhythm effect. The collection of the samples was done using a Salivette® (Sarstedt, Nuembrecht, Germany), by placing the swab under the tongue of the participant for 5 min and then centrifuging it immediately at 2600 rpm for 2 min. The supernatants were stored at -80ºC until their analysis.

SP and CGRP concentration was assessed using two specific commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) kits: Substance P Parameter Assay Kit (ref. KGE007; R&D systems, Minneapolis, MN, USA) and CGRP (Human) ELISA kit (ref. KA5439; Abnova, Taipei City, Taiwan). All the samples and controls were measured by duplicates and the plates were read at 450nm using the Full-Automatic Microplate Reader MB-580 (Shenzhen Huisong Technology Development Co., Ltd., Shenzhen, China) . Regarding the quantification of the concentration of neuropeptides, the absorbance value was first normalized by subtracting the value of each well from the absorbance measured in the non-sorbent control (well where there is no primary antibody or sample). The extrapolation of the concentration of the two neuropeptides was made using the straight-line equation.

7. Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and compared with: 1) T-test when there were only two groups to compare, using the paired test when the different measurements were performed on the same participant, or the unpaired test when two different groups had to be compared; and 2) One-way ANOVA tests were used when there were more than two groups to compare. In addition, a multiple comparison post test was performed to describe any differences between the groups when necessary.

Categorical data were expressed as relative and absolute frequencies and compared with the chi-square test. The correlations were determined with Spearman's correlation coefficient and a multiple linear regression test was performed when needed to describe whether any independent variable had a direct effect on the dependent variable.

In addition, a one-phase decay curve was used to describe the acute pharmacodynamic effects of the TRP agonists and thickeners in Chapter 2, following the model $Y=[Y_0\text{-Plateau}]\cdot e^{(-K\cdot X)}+\text{Plateau}$, followed by a Fisher's exact test to compare the proportion of patients whose prevalence of safety impairments improved by at least 30%, time to LVC and UESO by at least 100 ms, P2 latency shortened at least 20 ms and N2-P2 amplitude increased at least 2 µV.

Parametric or nonparametric tests were used when appropriate, after performing the D'Agostino & Pearson omnibus normality test in order to know if the variables had a normal distribution or not. Significant differences were considered when p-value<0.05.

All these statistical tests were performed using GraphPad Prism 6 and 9 (GraphPad Software, San Diego, CA, USA).

Finally, in order to assess the differences in brain source of pSEPS, the differences were computed voxel by voxel with a non-parametric t test using sLORETA software.

RESULTS

Chapter 1: Acute therapeutic effect of TRP agonists

Chapter 1.1: A randomized clinical trial on the acute therapeutic effect of TRPA1 and TRPM8 agonists in patients with oropharyngeal dysphagia

1. Study design

The study design included 4 visits:

a) On Visit 1, patients were screened with the V-VST for clinical signs of OD and clinical and demographic data were collected.

b) On Visit 2, 7 days later, the patient was randomized into a treatment group and given a VFS which consisted of: a control series (T0) with 5, 10 and 20mL boluses of nectar viscosity (181.92±1.75mPa·s at 50s-1); after two minutes, another series of 5, 10 and 20mL nectar viscosity boluses with the IP (T1) and, after five minutes, another series of nectar viscosity boluses containing the IP (T2) (Figure 1). All the boluses contained iodine radiopaque solution Omnipaque™ (GE Healthcare, Chicago IL, USA) diluted in water in a proportion 1:1.

c) On Visit 3, 3 to 7 days after the VFS, the first EEG (EEG1) was performed and then, between 3 and 7 days after the EEG1,

d) On visit 4, the second EEG (EEG2) was performed. During the EEGs, the pSEPs were evaluated before (control) and after two administrations of 35ml nectar viscosity boluses containing the IP or 1/2000 of ethanol as placebo (T1 and T2) the order of which was also randomized (Figure 1). At the end of each EEG visit, the patient was asked to evaluate the intensity and pleasantness of the administered treatment (IP or placebo) on a 0-10 scale to assess the palatability of each compound, and any sign of gut discomfort was registered with a gut comfort evaluation test.

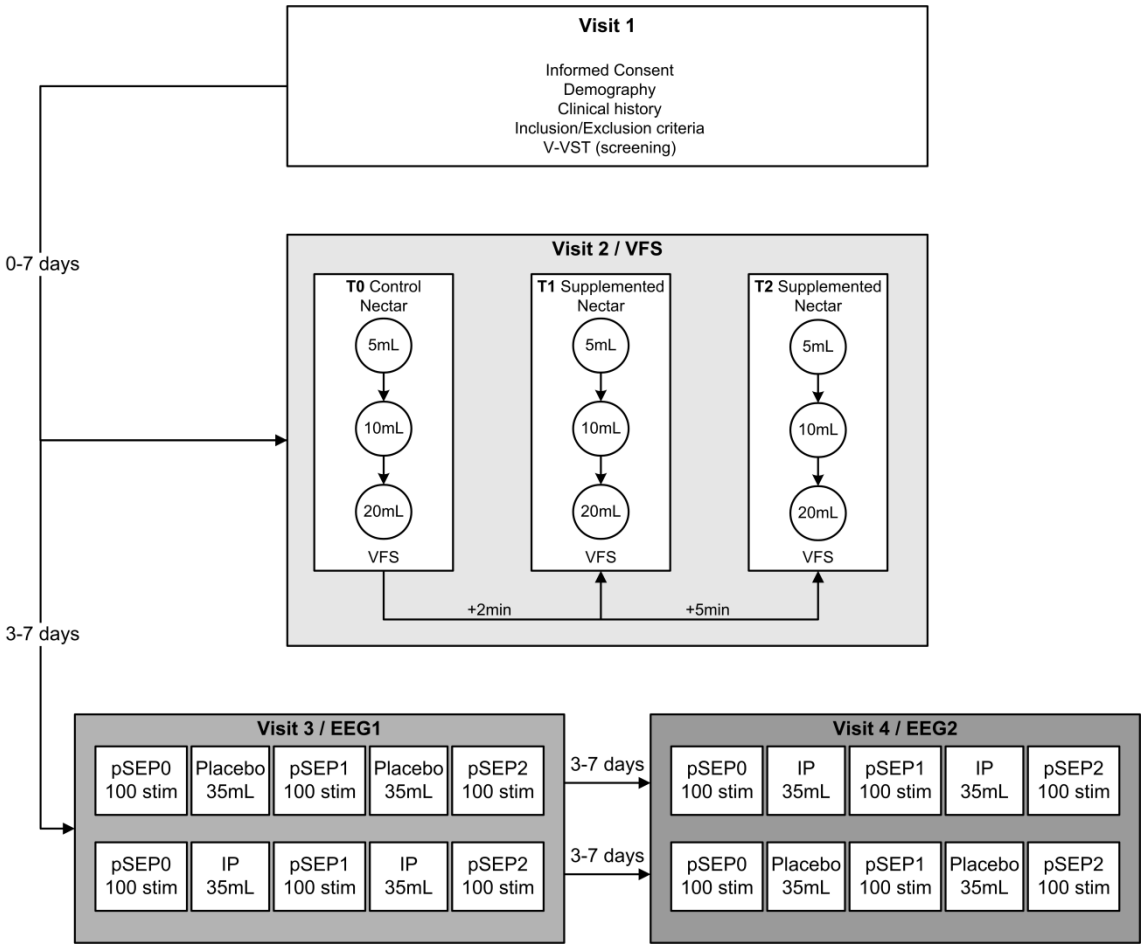

Figure 1: Study design including screening (Visit 1), videofluoroscopy (Visit 2) and EEG (Visit 3 and 4). Note that during VFS, each patient acts as his/her own control. VFS: videofluoroscopy; EEG: electroencephalogram; pSEP: pharyngeal sensory evoked potential; IP: investigated product.

2. *Study population*

For this study, 191 patients were assessed with the V-VST and 104 fulfilled the inclusion criteria and were referred for a VFS. Following the results of the VFS, 58 patients were finally included in the study and randomization into treatment groups: 21 for cinnamaldehyde-zinc (CIN-Zn), 21 for citral (CIT) and 16 for citral/isopulegol (CIT-ISO) (Figure 2).

Patients included in the study had a mean age of 79.8±7.6 years, were a similar number of men and women (55.2% women) and presented a high number of comorbidities (Charlson index) and mildly impaired functional status according to the Barthel index, without differences between groups (Table 1). The main cause of OD for the included patients was aging (56.9%) followed by neurodegenerative diseases (22.4%) and chronic stroke (20.7%) also without differences between groups (p=0.963).

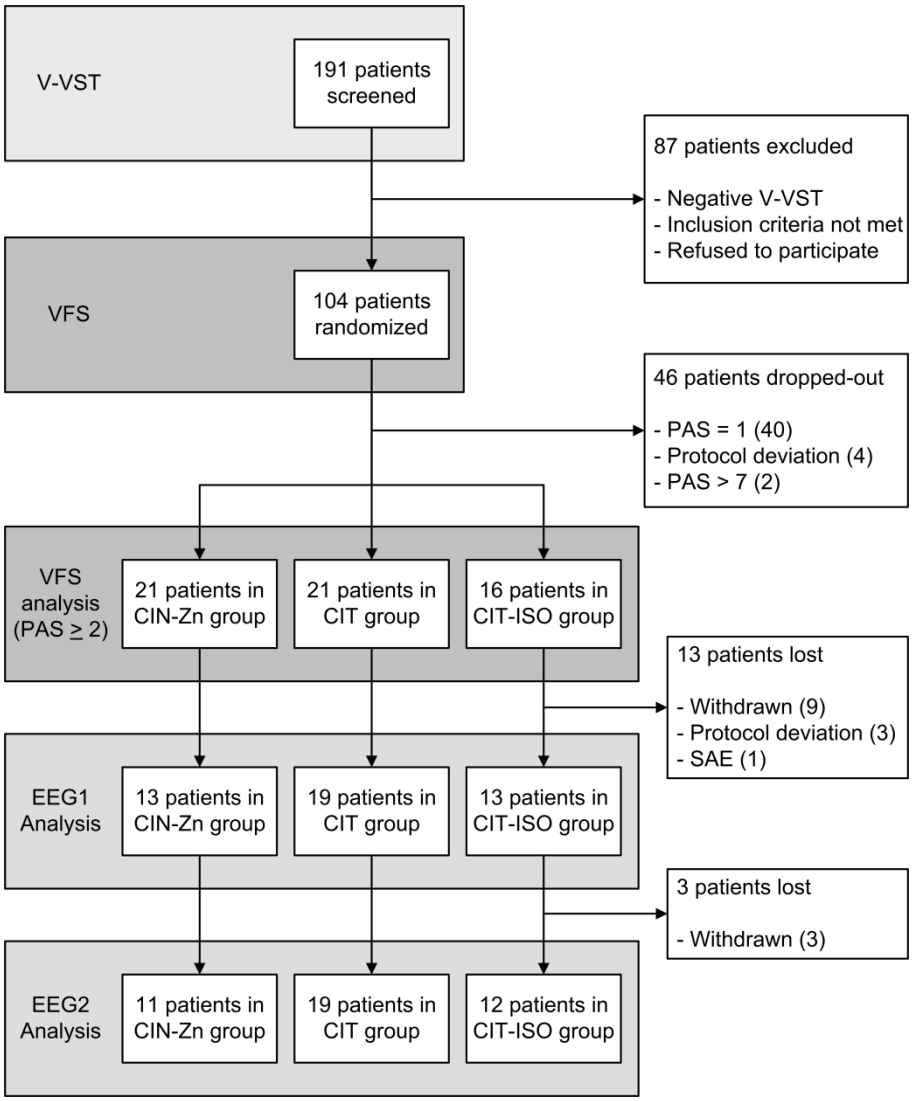

Figure 2: Patients flow chart. V-VST: volume-viscosity swallow test; VFS: videofluoroscopy; PAS: Penetration-Aspiration Scale; EEG: electroencephalogram; SAE: serious adverse event; CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group.

Table 1: Socio-demographic and clinical characteristics of the population in the three arms of the study.

	Total	CIN-Zn	CIT	CIT-ISO	P value
N	58	21	21	16	
Older (%)	56.9	61.9	57.1	50	0.769
Neurodegenerative disease (%)	22.4	19.0	23.8	25	0.895
Stroke (%)	20.7	19.0	19.0	25	0.882
Age (years)	79.8±7.6	81.6±7.6	78.0±7.5	79.8±7.9	0.233
Sex (% women)	55.2	61.9	57.1	43.6	0.532
Barthel index	66.6±33.4	75.8±33.8	58.5±30.7	63.2±35.6	0.444
Charlson index	2.8±1.6	2.8±1.3	3±2.0	2.6±1.6	0.792

Data are presented as mean±SD unless specifically stated. CIN-Zn: Cinnamaldehyde-Zinc treatment group; CIT: Citral treatment group; CIT-ISO: Citral-Isopulegol treatment group.

3. Baseline VFS signs and swallow biomechanics

All patients had VFS signs of impaired safety of swallow (PAS≥2) with penetrations into the laryngeal vestibule or aspirations at nectar control series; the mean PAS among patients was 3.86±1.43 and up to 8.62% patients presented aspirations. Regarding swallowing efficacy, 70.7% of the patients had oral residue and 56.9%, pharyngeal residue at nectar control series. Swallow response was also severely impaired in all patients, with delayed time to LVC when compared with normative data from healthy volunteers (HV) studied by our group under the same experimental conditions (398.6±151.2ms in patients vs 174.3±11.6ms in HV [119]) and time to UESO (287.6±114.7ms in patients vs 234.3±8.2ms in HV [119] at the same viscosity of the same thickening agent) (Table 2). The basal kinetic measures (mean bolus velocity and propulsion force) of a 5mL nectar bolus were similarly impaired between groups (Table 2).

Table 2. Swallow biomechanics characteristics of the study participants

	Total (n=58)	CIN-Zn (n=21)	CIT (n=21)	CIT-ISO (n=16)	P value
PAS	3.86±1.43	4.00±1.48	3.90±1.26	3.63±1.63	0.557
Oral residue	41 (70.7%)	14 (66.7%)	15 (71.4%)	12 (75.0%)	0.855
Pharyngeal residue	33 (56.9%)	13 (61.9%)	10 (47.6%)	10 (62.5%)	0.561
LVC (ms)	398.6±151.2	401.9±168.0	457.1±155.7	317.5±70.8	0.049
UESO (ms)	287.6±114.7	291.4±138.1	310.5±113.8	252.5±72.6	0.243
LVO (ms)	1121±333.9	1105±255.0	1141±431.5	1118±296.3	0.998
Mean velocity (m/s)	0.24±0.14	0.25±0.14	0.24±0.18	0.24±0.09	0.586
Propulsion force (mN)	307.6±425.3	338.9±425.7	323.6±561.0	245.4±142.4	0.759

Data presented as mean±SD except for oral and pharyngeal residue (%). PAS: penetration-aspiration scale; LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening; CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group.

There were no learning effects observed between the fixed sequential administration of boluses. PAS score and time to LVC worsened between the three control boluses which would not have been the case if there had been a learning effect: mean PAS was 2.3±1.7 in 5 mL, 2.6±1.7 in 10 and 3.3±1.4 in 20 mL (5 mL vs 20 mL: $p<0.0001$; 10 mL vs 20 mL: $p=0.019$); and mean LVC was 398.6±151.1 ms in 5 mL, 416.3±126.9 ms in 10 mL, and 497.4±166.9 ms in 20 mL (5ml vs 10 ml: $p=0.001$; 10 ml vs 20 ml: $p=0.001$).

4. Baseline neurophysiology

Pharyngeal sensory function was also impaired in the study participants. Patients had high sensory threshold to pharyngeal electrical stimuli (10.41±4.93mA in patients vs 6.0mA in HV [86]) while the mean tolerance threshold was also higher than the one previously found in HV (29.14±12.96mA in patients vs 24mA in HV [86]). The pSEP wave form in this study was

characterized by two negative (N1, N2) and two positive peaks (P1, P2) as we previously described in similar patients with OD [86]. The pSEP was also similarly impaired in our patients: the N2 peak latency was longer (228.27±46.60ms in patients vs 190ms in young HV [86]) and the N1-P1 and N2-P2 amplitudes were lower (3.20±2.21μV and 3.69±2.99μV in patients vs 6.6μV and 10.9μV in young HV [86]).

5. Effect of treatments on VFS signs and swallow biomechanics

Only the administration of CIN-Zn reduced the prevalence of signs of impaired swallowing safety during VFS. The PAS score was significantly reduced in the CIN-Zn group during the second administration (T2) of the IP (4.00±1.48 vs 3.19±1.81, p=0.009) (Figure 3) and the prevalence of patients with safe swallows increased from 52.38% to 80.95% at T1 (p=0.041). None of the TRP agonists significantly affected the prevalence of oral residues (from 70.7% to 81.0%, ns) or pharyngeal residues (from 56.9% to 67.2%, ns).

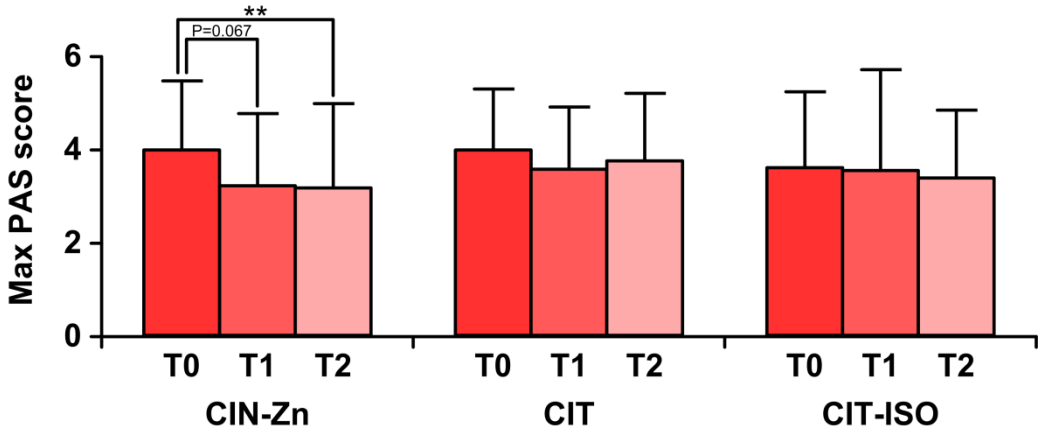

Figure 3: Representation of the mean±SD maximum PAS score registered during the V2 VFS. T0: control series; T1: first administration; T2: second administration; CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group; **: p-value<0.01.

The administration of both CIN-Zn and CIT significantly reduced the timing of oropharyngeal reconfiguration from a respiratory to a digestive pathway by shortening time to LVC and to UESO. Both series of the CIN-Zn group significantly reduced time to LVC (401.9±168.0ms vs 330.0±124.4ms, p=0.008 during T1 and vs 336.0±144.2ms, p=0.002 during T2) and time to UESO (291.4±138.1ms vs 238.0±98.4ms, p=0.023 during T1 and vs 238.0±132.0ms, p=0.007 during T2). In the CIT group, only the first administration significantly reduced time to LVC (457.1±155.7ms vs 405.7±117.5ms, p=0.023) and time to UESO (310.5±113.8ms vs 272.4±88.2ms, p=0.035). CIT-ISO significantly reduced time to UESO during both administrations (252.5±72.6ms vs 215.0±78.5ms, p=0.038 during T1 and vs 218.7±78.4ms, p=0.017 during T2) (Figure 4).

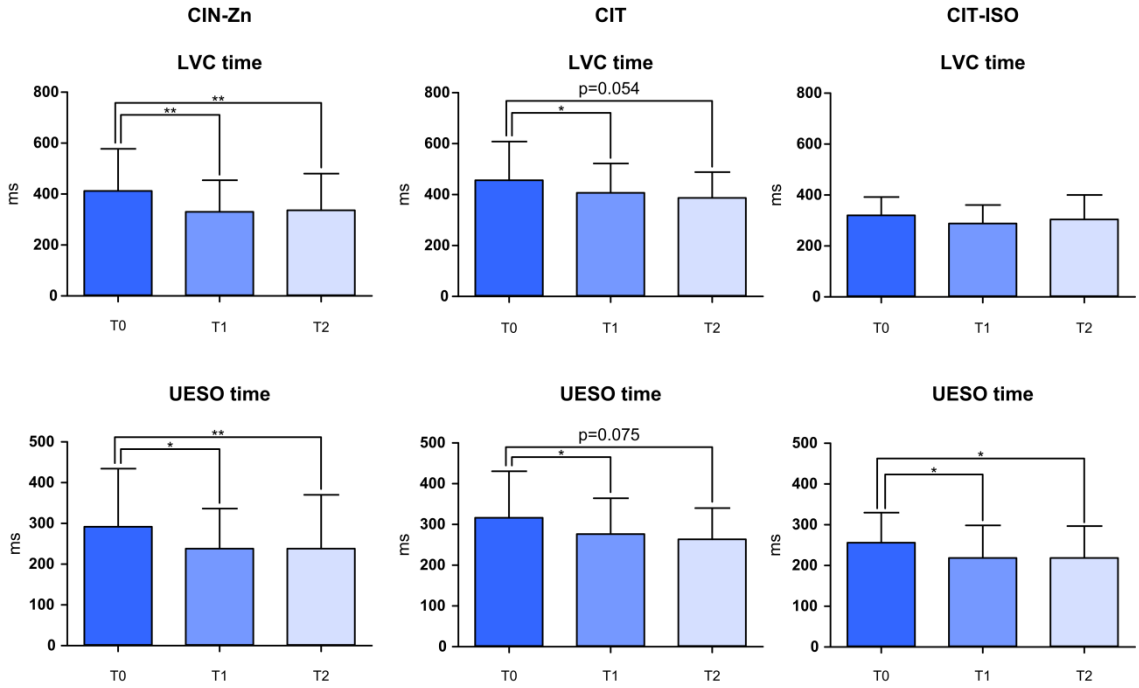

Figure 4: Effect of TRP agonists on the mean±SD timing of the swallow response: LVC and UESO. LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening; T0: control series; T1: first administration; T2: second administration; CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group; *: p-value<0.05; **: p-value<0.01.

6. *Effect of treatments on swallow neurophysiology*

6.1. *Pharyngeal sensory evoked potentials*

Baseline data on the pSEP were similar among the 3 groups (T0; p>0.05 for all comparisons). The administration of CIN-Zn significantly reduced the latency of the P2 peak and the amplitude of the N2-P2 peaks of the pSEP; moreover, this effect was significantly greater than the small changes caused by placebo in these patients. Both pSEPs registered after CIN-Zn administration presented a shorter P2 latency compared with the baseline (374.0±45.0ms vs 350.2±44.7ms, p=0.050 in T1 and 332.8±44.1ms, p=0.005 in T2). The pSEP registered after the second CIN-Zn administration presented a significantly reduced amplitude of the N2-P2 peaks compared with the baseline pSEP (5.88±4.40μV vs 3.74±2.45μV, p=0.005); this effect was not observed when administering placebo to the same patients.Among the other peaks and treatment groups, only the PSEP registered after the second CIT administration presented a significantly reduced amplitude of the P1-N2 peaks (2.93±2.57μV vs 2.48±2.64μV, p=0.042) (Figure 5).

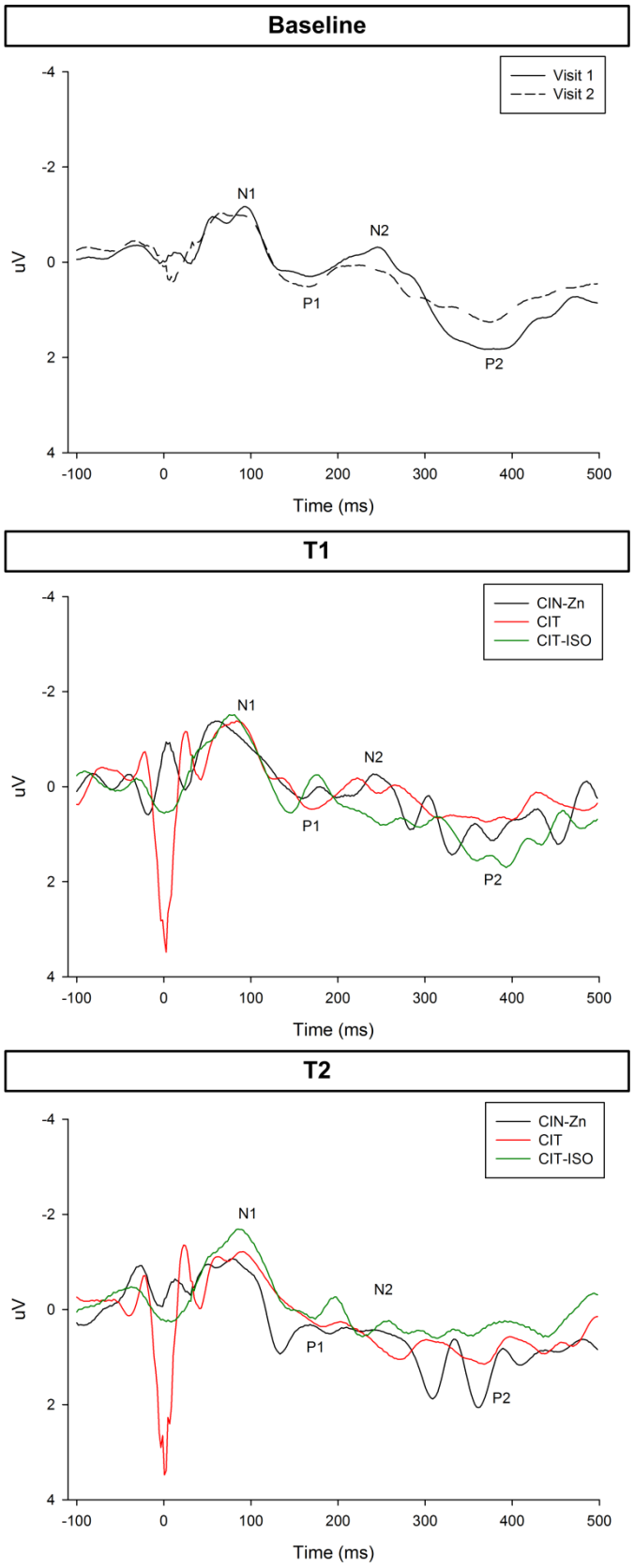

Figure 5: Pharyngeal sensory evoked potentials traces obtained at Cz for each treatment at baseline, T1 and T2 after pharyngeal electrical stimulation. Deflection at time point 0 corresponds to stimulus artifact. T1: first administration; T2: second administration; CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group; µV: microvolts.

6.2. Neurotopography

At T0, the source cortical activation areas through sLORETA analysis (Figure 6) agreed with our previously reported studies on older patients with OD[86], showing bi-hemispheric activation of prefrontal and anterior temporal cortices in early peaks (N1 and P1) and activation of parietal and posterior cingulate cortices in late peaks (N2 and P2).

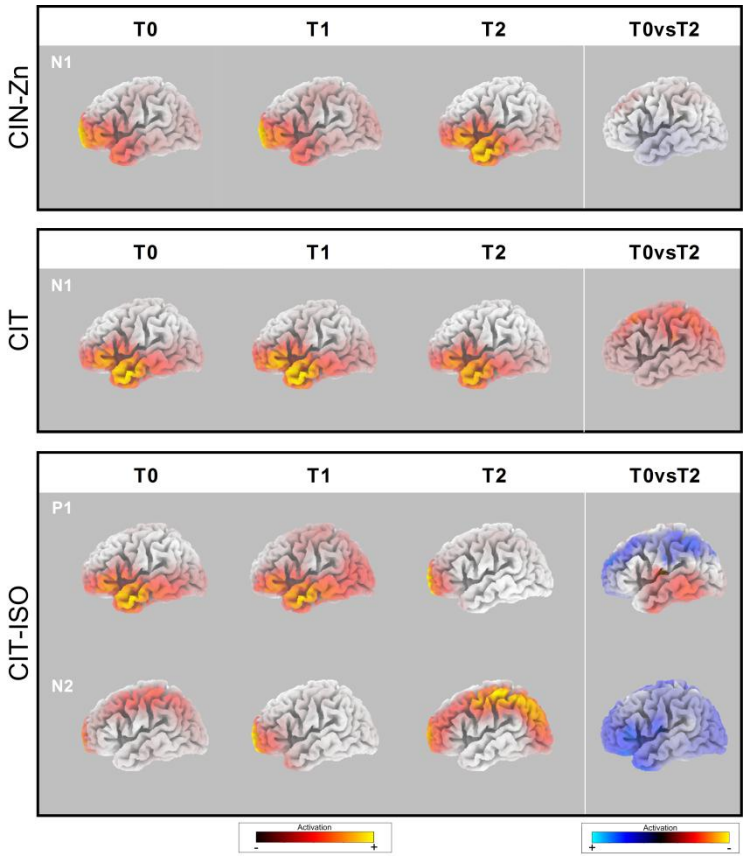

Figure 6: Representation of the LORETA source activity for each TRP agonist (CIN-Zn, CIT and CIT-ISO) at the three time points (T0, T1 and T2) evaluated in this study, and the comparison of T0 with T2 brain activation (T0vsT2). Colored voxels in the T0, T1 and T2 columns represent the most activated areas (red and yellow, increased activation), while the last column shows areas of significant voxel-wise differences (blue, increase; red, decrease) after correction for multiple comparisons. CIN-Zn: Cinnamaldehyde-Zinc group; CIT: Citral group; CIT-ISO: Citral-Isopulegol group; T0: baseline; T1: first administration; T2: second administration.

Temporary significant changes on source cortical activation occurred at T1 (with respect to T0) with all three treatments (Figure 7) but none of them remained at T2: Increased activation was shown in frontal gyri (Cin-Zn, CIT and CIT-ISO), precuneus (CIT) and cingulate gyrus (CIT-ISO), and decreased activation was shown in inferior frontal gyrus (Cin-Zn), post-central gyrus (CIT) and precuneus (CIT-ISO). Persistent significant T2 changes (with respect to T0) in the source cortical activation showed as significant focal enhancements only for the transverse temporal gyrus with Cin-Zn (N1-peak) and for the superior frontal gyrus and insula with CIT-ISO (P1 and

N2-peaks) represented by deep blue color in the T0vsT2 panel, while no significant increased activation was found with CIT (Figure 6, Figure 7).

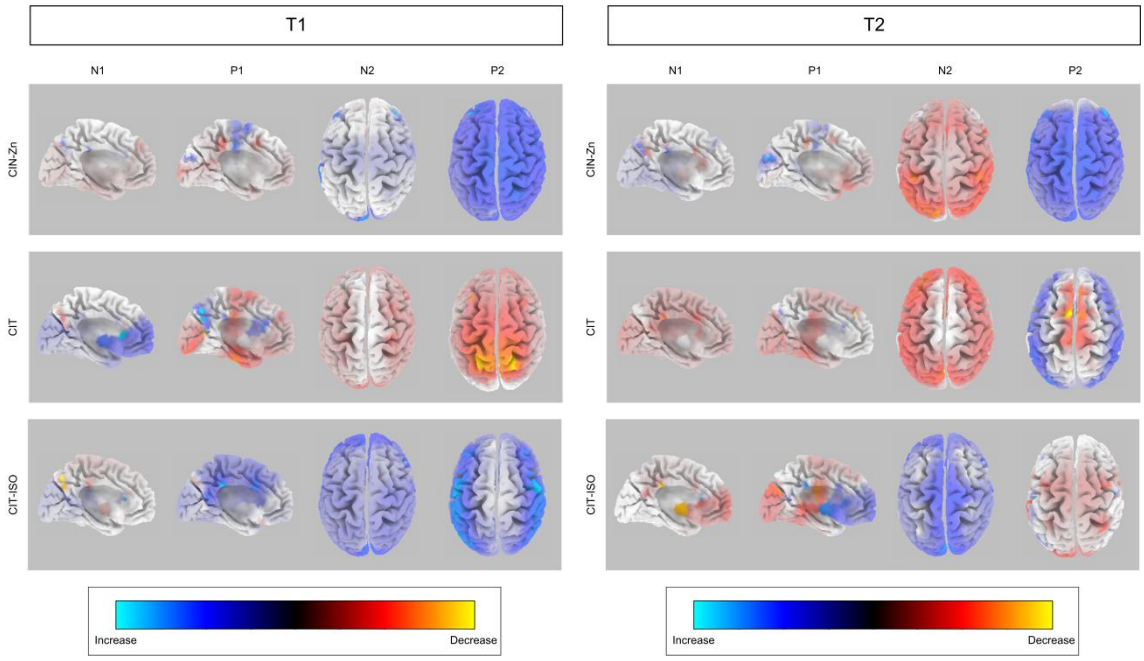

Figure 7: sLORETA comparison between baseline and T1 and T2 of each group. Note the increased activation in frontal gyri (CIN-Zn, CIT and CIT-ISO) and precuneus (CIT) after the first application of the treatments and the enhanced activation of the transverse temporal gyrus (CIN-Zn) after the second. T1: first administration; T2: second administration; CIN-Zn: cinnamaldehyde-zinc groups; CIT: Citral group; CIT-ISO: citral-isopulegol group.

7. Palatability of the treatments

Patients rated the IPs on a 0-10 scale as mildly intense compared with the placebo, although only the CIT group achieved significance (2.62±2.48 with placebo vs 5.50±2.84 with CIT, p=0.002). Regarding pleasantness, only CIT administration was perceived as less pleasant than placebo, but this difference was not significant (5.38±2.77 with placebo vs 4.35±2.76 with CIT, p=0.057). The treatment with the lowest prevalence of registered gut discomfort was CIN-Zn but there were too few to find significant differences between treatments.

8. Safety of the Treatment

During the study, twelve patients reported minor adverse events of six kinds. No relation was found between these and the IP except for pharyngeal irritation declared as possibly related to CIT-ISO. The most frequent event (75%) was gastrointestinal disorders (diarrhea and vomiting) related to the contrast agent used during VFS. No severe adverse events were reported.

Chapter 1.2: Effect of Aging, Gender and Sensory Stimulation of TRPV1 Receptors with Capsaicin on Spontaneous Swallowing Frequency in Patients with Oropharyngeal Dysphagia: A Proof-of-Concept Study

1. Study design

This study had two parts:

a) For the observational study, spontaneous swallowing frequency (SSF) was measured during 10 minutes in healthy volunteers (HV) and post-stroke patients with OD (PSD). All participants were requested to avoid body and head movements and talking during the experiment (Figure 1).

b) For the study on the effect of capsaicin on SSF, PSD patients received TRPV1 stimulation treatment using a 10µM capsaicin solution administered orally (4 bolus of 5ml) after the first 10-min SSF recording. In the intervals between capsaicin intakes, patients were requested to make dry swallows (2 to 4). A second 10-min SSF recording was made following the capsaicin treatment (Figure 1).

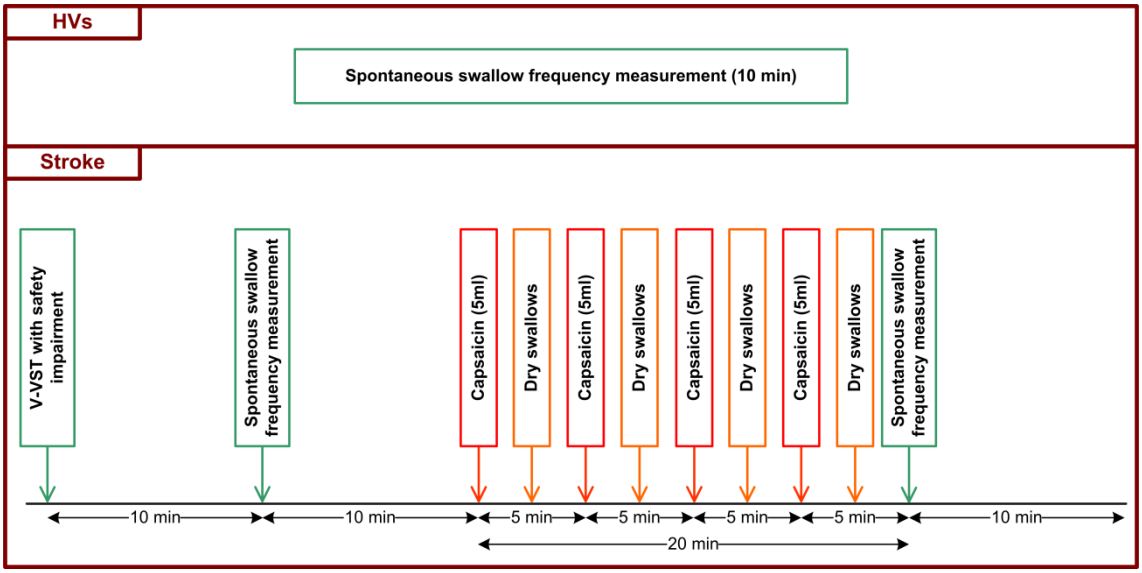

Figure 1. Study design of the two populations included in the study: HV at the top, post-stroke patients at the bottom.

2. Study population

A total of 141 healthy volunteers were included in the exploratory study and divided as follows: 50 in GI (28.08±5.84 years, 54.00% women), 49 in GII (50.08±4.71 years, 51.02% women) and 42 in GIII (63.38±7.92 years, 47.62% women).

Regarding the PSD patients, a total of 17 PSD patients (74.94±11.43 years, 41.2% women) were included in the study, 11 following an acute stroke and 6 patients with a chronic stroke (≥ 3 month from the stroke onset). Mean Rankin was 3.35±0.93, Barthel index of 69.41±27.43 and NIHSS of 6.59±5.23. The main type of stroke was ischemic (90.10%) and most of them were located in the left hemisphere (43.75%). According to the Oxford classification, 18.75% had a total anterior circulation infarct, 43.75% partial anterior circulation infarct, 6.25% lacunar infarct and 31.25% had posterior circulation infarct. V-VST results showed that 100% of PSD patients had signs of impaired safety of swallow and 82.35% also had efficacy impairment signs. Liquid was the unsafest viscosity (70.59%), followed by nectar (29.41%) and pudding was the safest one (5.88%) (p<0.0001). Regarding the efficacy impairments, pudding viscosity presented the highest rate of pharyngeal residue (76.47%) when compared to nectar (58.82%) and liquid (25%) (p=0.01). Indications for VFS were personalized according to the evolution and clinical setting and mean PAS score was 4.00±2.58.

3. *Basal SSF in HV and PSD patients*

The mean SSF in all HV was 0.8±0.5 swallows/min. Analyzing the results according to the age of the participants, SSF in the GI was 1.03±0.62 swallows/min and was significantly reduced in GII (0.73±0.50 swallows/min; p=0.0385) and GIII (0.50±0.31 swallows/min; p<0.0001) (Figure 2a). In addition, there was a moderate significant negative correlation between age and SSF (r=-0.3810, p<0.0001) (Figure2b).

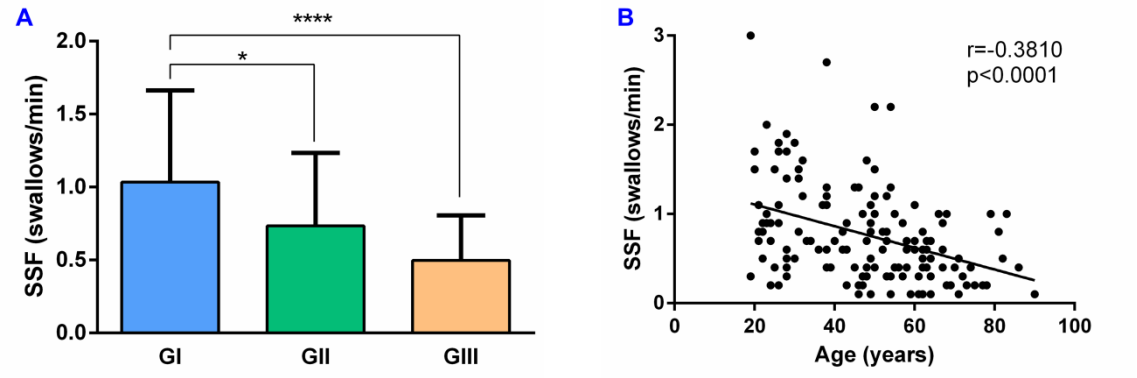

Figure 2. SSF and its correlation with age in HV: a) SSF results by age groups; b) correlation between SSF and age. SSF: spontaneous swallowing frequency; GI: 18-39 years age group; GII: 40-59 years age group; GIII: ≥60 years age group; *: p<0.05; ****: p<0.0001.

Regarding the effect of gender, the mean SSF in women was 0.82±0.55 swallows/min and in men 0.72±0.53 swallows/min (p=0.1846). When comparing these results between age groups, in women there were significant differences between GI (1.01±0.57 swallows/min) and GIII (0.52±0.36 swallows/min; p=0.0067) groups but not with GII (0.86±0.56 swallows/min) (Figure

3a). In men, however, there was a significant reduction in GII (0.61±0.38 swallows/min; p=0.0345) and GIII (0.48±0.25 swallows/min; p=0.0029) when compared to GI (1.06±0.67 swallows/min) (Figure 3c). A negative correlation between SSF and age was also found in women (r=-0.3534, p=0.0023) (Figure 3b) and men (r=-0.3858, p=0.0011) (Figure 3d). However, according to the multiple linear regression test, our data show that SSF is only affected by age (F(1, 138)=24.55; p<0.0001) but not by gender (F(1, 138)=1.347; p=0.2479).

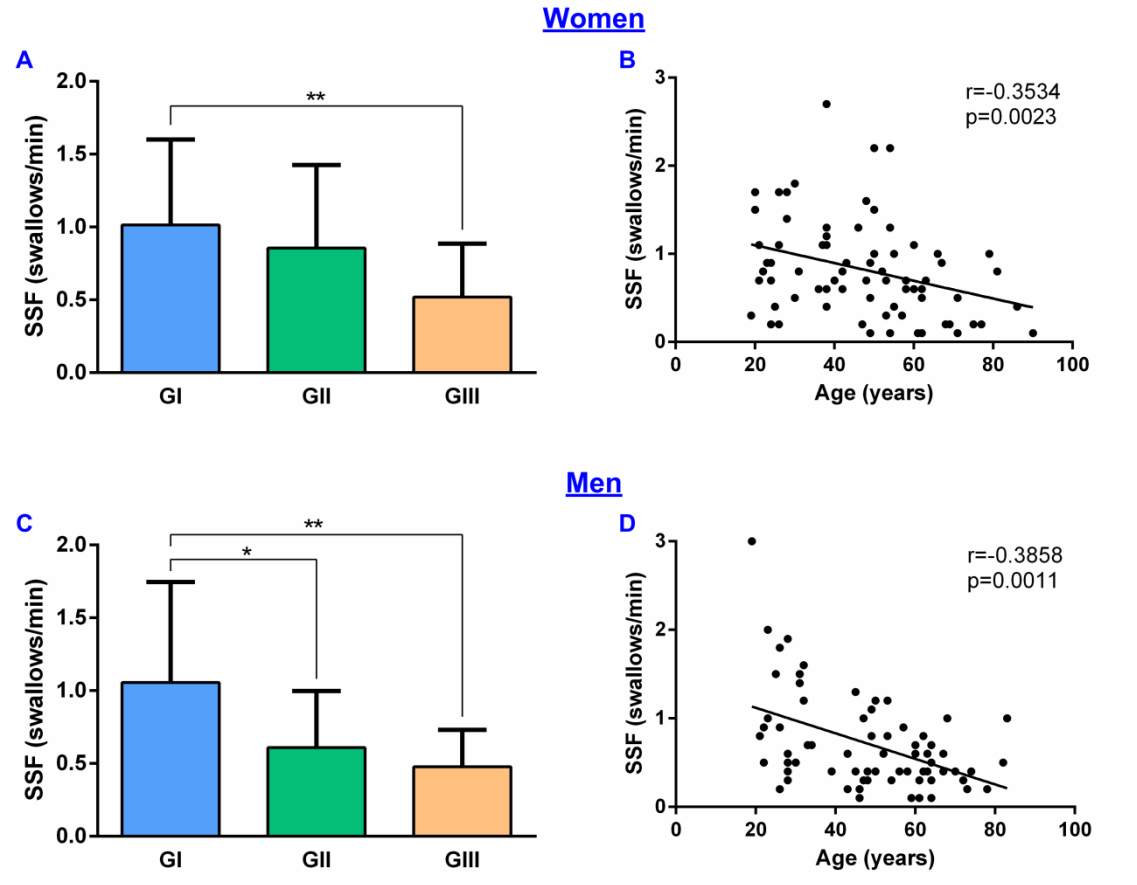

Figure 3. Gender effect on SSF: a) SSF results by age groups in women; b) correlation between SSF and age in women; c) SSF results by age groups in men d) correlation between SSF and age in men. SSF: spontaneous swallowing frequency; GI: 18-39 years age group; GII: 40-59 years age group; GIII: ≥60 years age group; *: p<0.05; **: p<0.01.

Finally, PSD patients showed a SSF of 0.41±0.32 swallows/min without significant differences when compared to the same age group in HV (GIII) (p=0.3112).

4. Basal EMG metrics

In HV, the mean amplitude was 0.70±0.61 volts; the mean delta T, 1.82±0.39 seconds and the mean AUC, 0.26±0.37 volts·seconds. Taking into account the three age groups, we found a significant reduction only in the duration of the EMG signal (delta T) that was reduced by age between GI (1.88±0.20 seconds) and GIII (1.74±0.14 seconds; p=0.0178) (Figure 4a). Regarding

the effect of gender, significant differences between men and women were observed. Women showed a reduction in the amplitude (GII: 0.44±0.11 volts, p=0.0158; GIII: 0.46±0.13 volts, p=0.0500; vs GI: 0.55±0.11 volts) with age (Figure 4b) while in men a reduction in delta T (GI: 2.04±0.19; GII: 1.81±0.15 seconds, p=0.0071 (vs GI); GIII: 1.66±0.05, p<0.0001 (vs GI) and p=0.0439 (vs GII)) was observed (Figure 4c). No significant differences were observed for AUC.

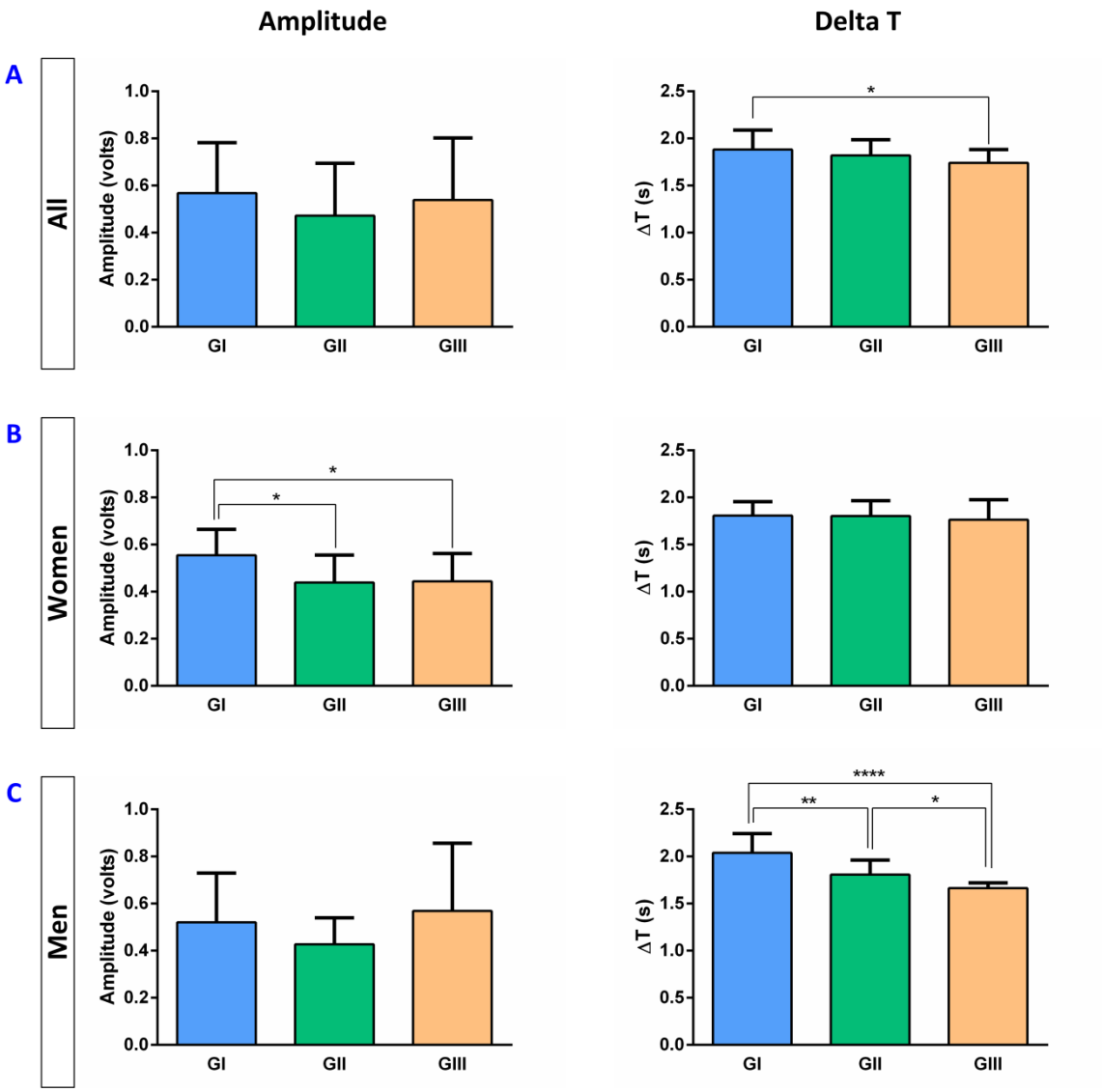

Figure 4. EMG metrics (Amplitude and Delta T) in all HV (a), women (b) and men (c). ΔT: Delta T; s: seconds; GI: 18-39 years group; GII: 40-59 years group; GIII: ≥60 years group; *: p<0.05; **: p<0.01; ****: p<0.0001.

We also found that PSD patients presented an increased amplitude (0.89±0.55 volts, p=0.0029) (Figure 5a), delta T (2.38±0.78 seconds, p=0.0004) (Figure 5b) and AUC (0.50±0.43 volts-seconds, p<0.0001) (Figure 5c) when compared to HV in GIII group with similar age.

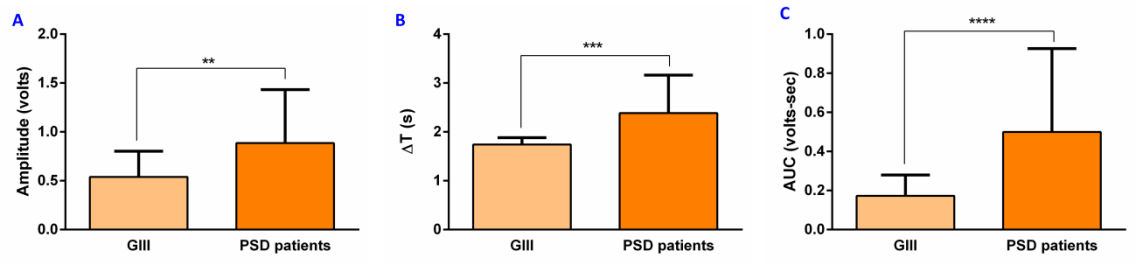

Figure 5. EMG metrics in PSD patients and GIII group: a) amplitude, b) Delta T and c) AUC. ΔT: Delta T; AUC: area under the curve; s: seconds; GIII: ≥60 years group; PSD: post-stroke dysphagia; **: p<0.01; ***: p<0.001; ****: p<0.0001.

5. *Effect of TRPV1 stimulation on SSF and EMG metrics in PSD*

After TRPV1 stimulation with capsaicin (10μM), PSD patients showed a significant increase in SSF (0.81±0.51 swallows/min, p=0.0003) compared to the frequency registered before treatment (0.41±0.32 swallows/min) (Figure 6a, Figure 7). However, no significant changes in the other EMG metrics were observed: amplitude pre-treatment, 0.89±0.55 volts vs post-treatment, 1.24±0.98 volts (Figure 6b), p=0.1503; delta T pre-treatment, 2.38±0.78 seconds vs post-treatment, 2.30±0.66 seconds, p=0.7831; AUC pre-treatment, 0.50±0.43 volts·second vs post-treatment, 0.59±0.57 volts·second, p=0.8400.

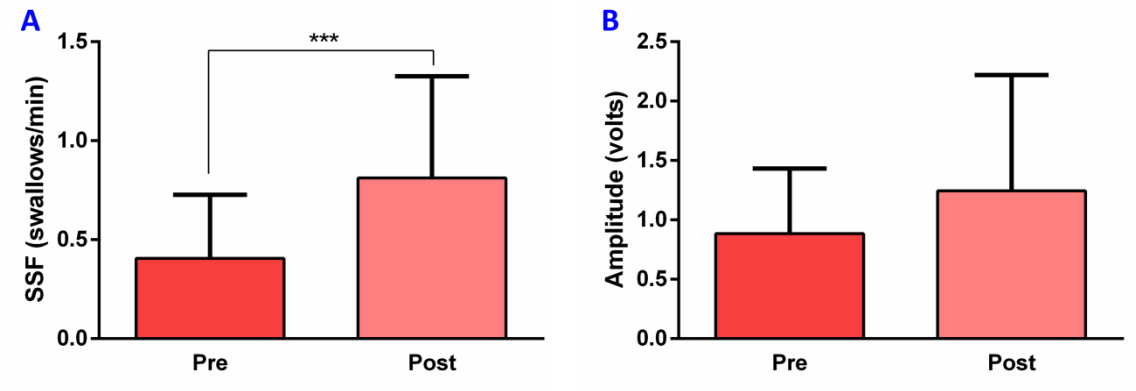

Figure 6. Capsaicin effect on SSF and EMG metrics in post-stroke dysphagia patients: a) SFF, b) Amplitude. SSF: spontaneous swallowing frequency; s: seconds; pre: pre-treatment; post: post-treatment; ***: p<0.001.

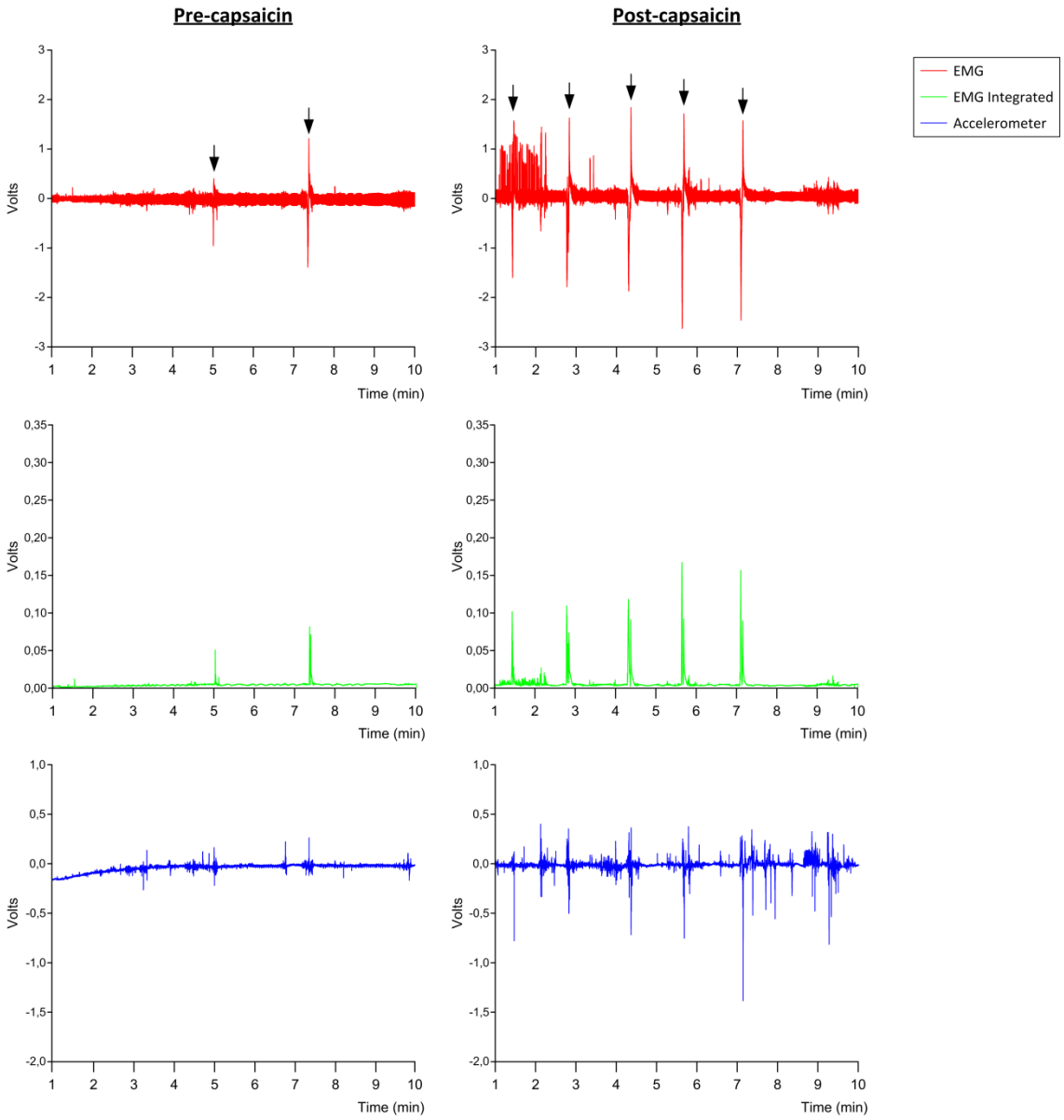

Figure 7. Pre and post-capsaicin stimulation SSF registration. Red line represents the electromyography (EMG) signal; Green line represents the EMG integrated signal; Blue line represents the accelerometer movement; Black arrows point to each swallow registered.

Chapter 2: A comparative study on the effect of acute pharyngeal stimulation with TRP agonists on the biomechanics and neurophysiology of swallow response in patients with oropharyngeal dysphagia

1. Study design

Observational retrospective study that evaluates and compares the biomechanical and neurophysiological effects of TRP agonists and TPs. All the studies collected clinical and socio-demographic data, including the cause of OD and the functional (Barthel Index) and nutritional status (Mini nutritional assessment short form -MNA-sf-). The pre-treatment and post-treatment prevalence of visuoperceptual signs of impaired safety and efficacy of swallow and biomechanical evaluation of swallow with VFS was also collected in those studies where the therapeutic effect of TRP agonists was described. Finally, we gathered neurophysiological information from studies that included the assessment of pSEPs to describe the effect of the treatments on swallowing neurophysiology.

The therapeutic effect of each compound (TP vs TRP agonist) was evaluated according to the effect that each treatment had on the VFS signs of safety (prevalence of penetrations and aspirations) and efficacy (prevalence of oral and pharyngeal residue), the biomechanics of swallowing (times to LVC, UESO and mean bolus velocity) and the neurophysiology of swallowing (latency and amplitude of pSEP peaks). The comparison was made as follows: in the TRP agonists studies, the effect on biomechanics and neurophysiology of swallowing was calculated as the % of change of each parameter between T0 and T1 and between T0 and T2, while the VFS signs were evaluated taking into account the three complete nectar series (5, 10 and 20 ml in T0, T1 and T2). Regarding TP studies, the prevalence of VFS signs was calculated taking into account the complete nectar and pudding series; the effect on biomechanics was evaluated as the % of change between 5ml nectar and 5 ml pudding; and the effect on neurophysiology was taken as the % of change between the first and last pSEP recorded (Figure 1).

The one-phase decay curves enabled the results to be differentiated into three groups according to their pharmacological effect, taking into account the ED50 value: high, intermediate and low (Table 1).

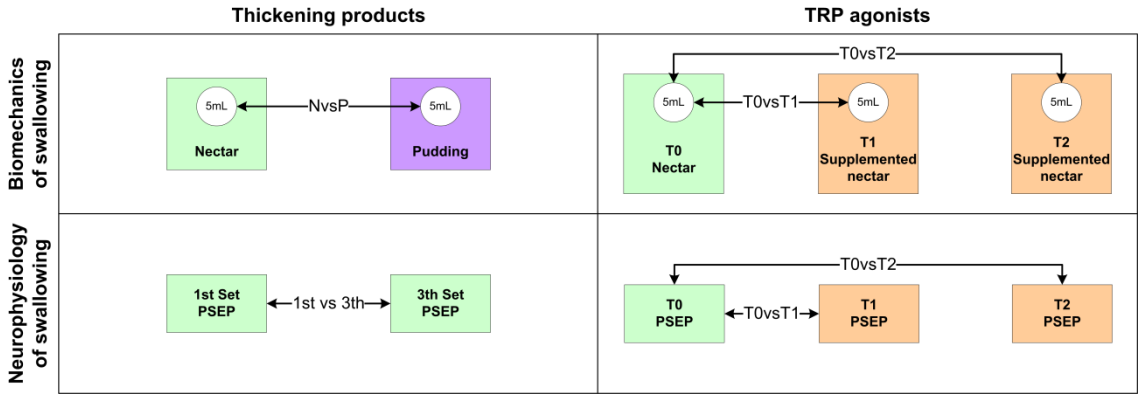

Figure 1: Data analysis. N: nectar; P: pudding; T0: basaline; T1: first time point; T2: second time point; PSEP: pharyngeal sensory evoked potential

Table 1: Criteria to determine the intensity of the therapeutic effect.

Variable	Therapeutic effect
Signs of safety impairment	High: > 30% Intermediate: 29-10% Low: < 10%
Time to LVC and UESO	High: > 100ms Intermediate: 99 - 50ms Low: < 50ms
P2 peak latency	High: > 20 ms Intermediate: 19 - 10ms Low: < 10ms
N2-P2 amplitude	High: > 2µV Intermediate: 2 - 1µV Low: < 1µV

LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening.

2. Study population

A total of 347 patients with OD (50.7% men; 78.3±6.3 years) were evaluated using VFS (178 in the TRP stimulation studies and 169 in the TP studies). Etiology of OD was a consequence of: aging (34.0%) chronic post-stroke (51.0%), and neurodegenerative diseases (15.0%). Table 2 specifies the characteristics of each group.

Table 2. Demographic characteristics of study groups.

	TOTAL	CAPS 150 µM	CAPS 10 µM	PIPE 1 mM	PIPE 150 µM	MENT 10 mM	MENT 1 mM	CIN-Zn	CIT	CIT-ISO	MS TP	XG TP	p-value
n	329	33	7	20	20	20	20	21	21	16	33	118	
Age (years)	78.2±6.5	75.9±1.9	83.5±6.3	75.1±3.3	76.6±2.4	78.2±8.2	77.6±8.4	81.6±7.6	78.0±7.5	79.8±7.9	73.9±2.2	74.4±12.4	>0.999
Sex (% men)	47.8	60.6	57.1	40	45	40	55	38.1	42.9	56.4	48.5	54.2	0.708
Barthel Index	71.9±26.4	n/a	70±33.7	74.2±7.9	78.0±6.9	75±33.9	80.3±28.5	75.8±33.8	58.5±30.7	63.2±35.6	n/a	n/a	0.999
MNA-sf	11.6±3.9	n/a	9.5±2.9	n/a	n/a	18.0±7.3	9.5±2.63	n/a	n/a	n/a	n/a	9.7±2.8	0.823
OD Etiology (%)													
Aging	41.6	30.3	57.1	60	55	n/a	n/a	62	57.1	50	30.3	34.17	**0.035**
Stroke	42,5	45.5	28.6	40	25	n/a	n/a	19.0	23.8	25	45.5	55	**0.004**
ND	15.8	24.3	14.3	0	20	n/a	n/a	19.0	19.0	25	24.2	10.83	**0.018**

CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-ZN: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; ND neurodegenerative; MNA-sf: mini nutritional assessment-short form.

3. The effect on VFS signs

3.1. Safety of swallow

3.1.1. Thickening products

Increasing fluid viscosity from liquid to nectar increases the prevalence of patients that can swallow safely to up to 56% using XG-based TP and to up to 80% using MS-based TP. In addition, both types of TPs evaluated in this study caused a strong and significant reduction in the prevalence of signs of safety impairment when fluid viscosity was increased from nectar to pudding: modified starch TP by 78.05%, $p<0.0001$; and xanthan gum TP by 73.5%$p<0.0001$ (Figure 2a).

3.1.2. TRP agonists

When comparing acute TRP agonist stimulation with its own non-supplemented nectar control, capsaicin significantly reduced the prevalence of safety impairments by 44.18% at T1 ($p=0.0241$) and 50% ($p=0.0089$) at T2 in a concentration of 150µM and by 30.32% at T2 ($p=0.0478$) in a concentration of 10µM. Piperine also reduced the prevalence up to 56% at T1 ($p=0.0024$) (Figure 2a).

There were no significant differences in the therapeutic effects of TRP agonists when comparing T1 and T2.

3.1.3. Thickening products vs TRP agonists

Increasing fluid viscosity from nectar to pudding with TPs caused a significantly greater reduction in the prevalence of signs of safety impairment than supplementation of nectar with any of the TRP agonists tested. Capsaicin at a concentration of 150µM and piperine at a concentration of 1mM at T1 showed a stronger therapeutic effect than the other agonists (Table 3).

Table 3: Results of the comparison of the therapeutic effect on VFS signs.

		CAPS 150µM		CAPS 10µM		PIPE 1mM		PIPE 150µM		MENT 10mM		MENT 1mM		CIN-Zn		CIT		CIT-ISO		MS TP	XG TP
		T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	P-N	P-N
CAPS 150µM	T1-C			**		*		*		****		****		****		****		*		****	****
	T2-C				Ns		****		****		****		****		****		****		****	****	****
CAPS 10µM	T1-C					***		*		Ns		Ns		Ns		Ns		Ns		****	****
	T2-C						Ns		Ns		Ns		Ns		Ns		Ns		Ns	*	Ns
PIPE 1mM	T1-C							***		****		****		****		****		**		***	**
	T2-C								Ns		****		Ns		Ns		****		***	****	****
PIPE 150µM	T1-C									****		*		***		****		Ns		****	****
	T2-C										****		*		Ns		****		***	****	****
MENT 10mM	T1-C											****		****		****		****		****	****
	T2-C												****		**		*		Ns	****	****
MENT 1mM	T1-C													*		****		Ns		****	****
	T2-C														**		****		****	****	****
CIN-Zn	T1-C															***		Ns		****	****
	T2-C																****		Ns	****	****
CIT	T1-C																	***		****	****
	T2-C																		*	****	****
CIT-ISO	T1-C																			****	****
	T2-C																			****	****
MS TP	P-N																				Ns

CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; MS TP: modified starch thickening product; XG TP: xanthan gum thickening product; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; Ns: not significant.

3.2. Efficacy impairments

The effect of TP and TRP agonists on the efficacy of swallow are summarized in Figure 2b. Neither TRP agonists nor TPs had a significant effect on signs of efficacy impairment.

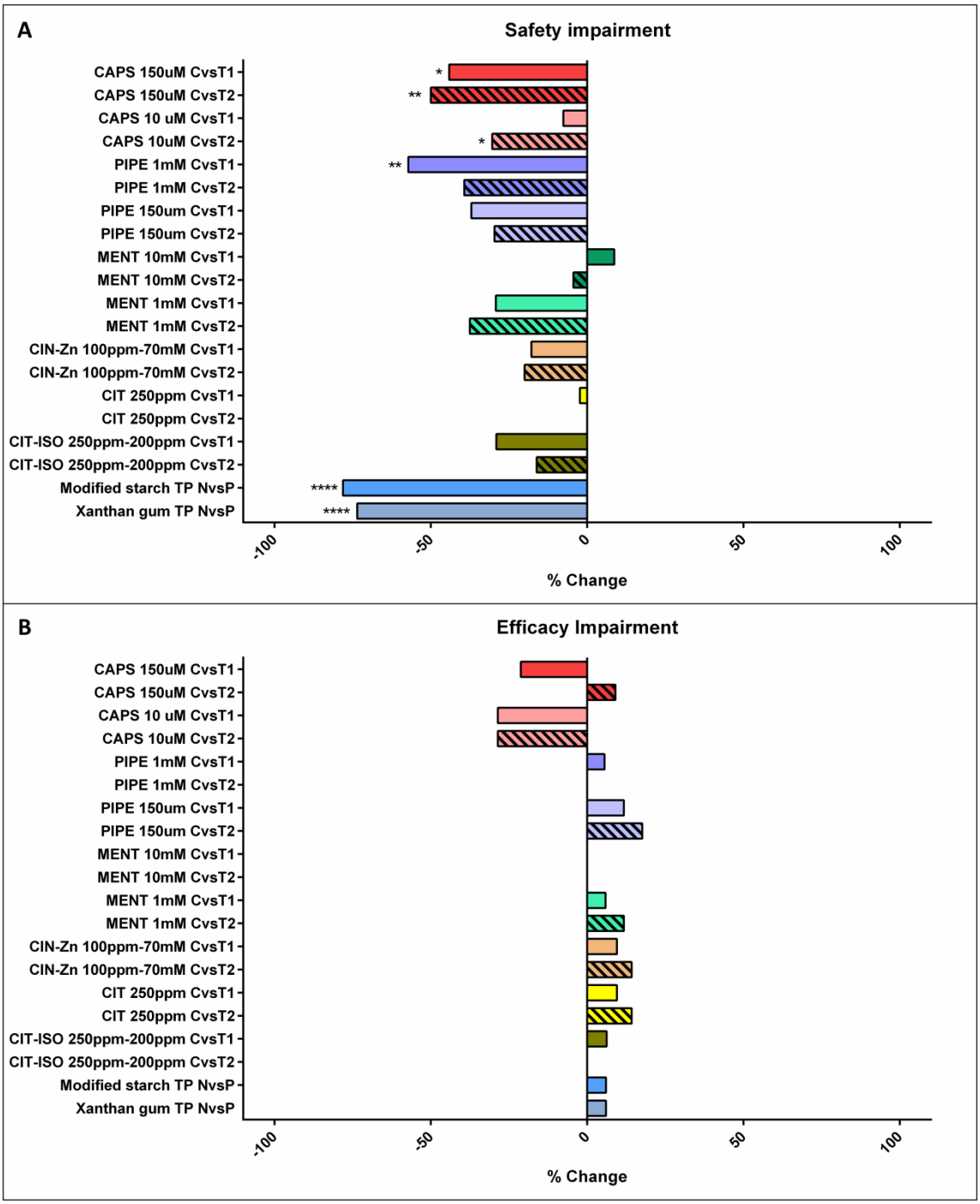

Figure 2: Normalized effect of compensatory and active treatments on signs of safety (A) and efficacy (B). CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; *: $p<0.05$; **: $p<0.01$; ****: $p<0.0001$.

4. The effects on the biomechanics of the swallow response

4.1. The effect on the timing of OSR

4.1.1. Thickening products

Increasing the viscosity from nectar to pudding prolonged the timing of the OSR. Modified starch TP significantly increased the time to UESO by 24.1% (p=0.04), while xanthan gum TP increased both the time to LVC by 25.8% (p=0.02) and to UESO by 35.5% (p=0.03) (Figure 3a,b). In addition, as shown in Table 4 and 5, both TPs had significant differences with most TRP agonists.

4.1.2. TRP agonists

The three types of TRP agonists (TRPV1, TRPA1 and TRPM8 agonists) significantly reduced the time to LVC, when compared with their own controls: capsaicin 150µM (T1: 27.6%, p=0.0006; T2: 11.9%, p=0.0499), piperine 1mM (T1: 19.5%, p=0.0117; T2: 16.8%, p=0.0061) and 150µM (T1: 27.0%, p=0.0003; T2: 13.5%, p=0.0120), menthol 10mM (T1: 17.0%, p=0.0364; T2: 18.0%, p=0.0102), CIN-Zn (T1: 17.9%, p=0.0057; T2: 16.4%, p=0.0009) and CIT (T1: 11.3%, p=0.0195; T2: 14.6%, p=0.0438) (Figure 3a). In addition, capsaicin 150µM and piperine in both concentrations reduced time to LVC significantly more than the other agonists (Table 4).

Regarding the time to UESO, TRPV1, TRPA1, and TRPA1-MB agonists also caused a significant reduction, the TRPV1 agonist capsaicin 150µM reduced it by 19.5% (p=0.0121) at T1, the TRPA1 agonist CIN-Zn by 18.9% (p=0.0213) at T1 and 18.8% (p=0.0027) at T2, the TRPA1 agonist CIT by 12.3% (p=0.0309) at T1, and the TRPA1-TRPM8 agonists CIT-ISO by 14.9% (p=0.0374) at T1 and 13.4% (p=0.0181) at T2 (Figure 3b). When comparing between them, TRP agonists with significantly higher effect on the reduction of time to UESO were CIN-Zn, capsaicin 150µM and CIT-ISO (Table 5).

There were no significant differences between T1 and T2 for time to LVC or UESO.

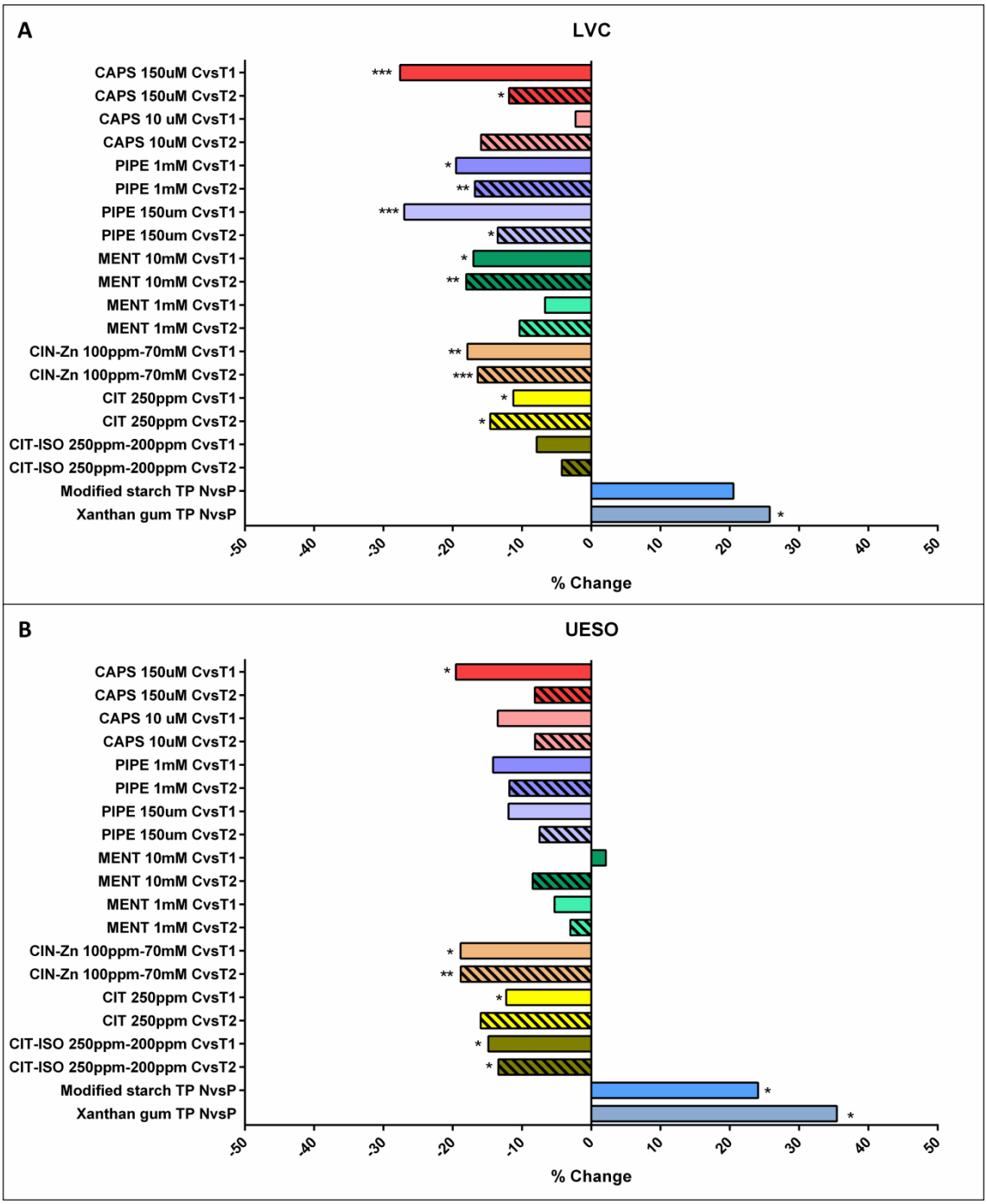

Figure 3: Normalized effect of compensatory and active treatments on the time to LVC (A) and to UESO (B). LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening; CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 4: Results of the comparison of the therapeutic effect on time to LVC.

		CAPS 150µM		CAPS 10µM		PIPE 1mM		PIPE 150µM		MENT 10mM		MENT 1mM		CIN-Zn		CIT		CIT-ISO		MS TP	XG TP
		T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	P-N	P-N
CAPS 150µM	T1-C			Ns		*		Ns		Ns		**		Ns		**		****		****	**
	T2-C				Ns		Ns		Ns		Ns		Ns		Ns		Ns		Ns		**	Ns
CAPS 10µM	T1-C					Ns		Ns		Ns		Ns		Ns		Ns		Ns		Ns	Ns
	T2-C						Ns		Ns		Ns		Ns		Ns		Ns		Ns		*	Ns
PIPE 1mM	T1-C							*		Ns		Ns		Ns		Ns		**		****	*
	T2-C								Ns		Ns		Ns		Ns		Ns		Ns		****	*
PIPE 150µM	T1-C									Ns		**		Ns		**		****		****	**
	T2-C										ns		Ns		Ns		Ns		Ns		****	*
MENT 10mM	T1-C											Ns		Ns		Ns		Ns		***	*
	T2-C												Ns		Ns		Ns		Ns		****	*
MENT 1mM	T1-C													Ns		Ns		Ns		***	Ns
	T2-C														Ns		Ns		Ns		****	*
CIN-Zn	T1-C															Ns		Ns		****	*
	T2-C																Ns		Ns		****	*
CIT	T1-C																	Ns		****	*
	T2-C																		ns	***	*
CIT-ISO	T1-C																			****	Ns
	T2-C																			*	Ns
MS TP	P-N																				Ns

CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; MS TP: modified starch thickening product; XG TP: xanthan gum thickening product; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; Ns: not significant.

Table 5: Results of the comparison of the therapeutic effect on time to UESO.

		CAPS 150µM		CAPS 10µM		PIPE 1mM		PIPE 150µM		MENT 10mM		MENT 1mM		CIN-Zn		CIT		CIT-ISO		MS TP	XG TP
		T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	T1-C	T2-C	P-N	P-N
CAPS 150µM	**T1-C**			Ns		Ns		Ns		*		**		Ns		Ns		Ns		****	*
	T2-C				Ns		Ns		Ns		Ns		Ns		*		Ns		Ns	****	*
CAPS 10µM	**T1-C**					Ns		Ns		Ns		Ns		Ns		Ns		Ns		*	Ns
	T2-C						Ns		Ns		Ns		Ns		****		Ns		*	****	*
PIPE 1mM	**T1-C**							Ns		Ns		Ns		Ns		Ns		Ns		****	*
	T2-C								ns		Ns		Ns		Ns		Ns		Ns	***	*
PIPE 150µM	**T1-C**									Ns		Ns		Ns		Ns		Ns		****	*
	T2-C										Ns		Ns		****		Ns		**	****	*
MENT 10mM	**T1-C**											Ns		Ns		Ns		Ns		*	ns
	T2-C												Ns		****		Ns		*	****	*
MENT 1mM	**T1-C**													Ns		Ns		*		****	Ns
	T2-C														Ns		Ns		Ns	Ns	Ns
CIN-Zn	**T1-C**															Ns		Ns		****	*
	T2-C																Ns		*	****	*
CIT	**T1-C**																	Ns		****	*
	T2-C																		Ns	****	*
CIT-ISO	**T1-C**																			****	*
	T2-C																			****	*
MS TP	**P-N**																				Ns

CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; MS TP: modified starch thickening product; XG TP: xanthan gum thickening product; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; Ns: not significant.

4.2. The effects on bolus velocity

An opposite effect on mean bolus velocity was also observed between TRP stimulants and increasing fluid viscosity with TP. Capsaicin 150µM at T1 (24.8%, p=0.0065), piperine 150µM at T1 (18.3%, p=0.0295), CIN-Zn at T2 (24.6%, p=0.0171) and CIT-ISO at T1 (27.8%, p=0.0419) and T2 (15.1%, p=0.0161) significantly increased mean bolus velocity. In contrast, increasing bolus viscosity from nectar to pudding with modified starch TP significantly reduced bolus velocity by 25.6% (p=0.0319).

When comparing T1 with T2 no significant differences were found.

5. The effects on neurophysiology

When comparing the latencies of the characteristic peaks of pSEPs before and after the acute treatments with TRP agonists, we only found significant differences in P2 peak latency with CIN-Zn treatment, which shortened it by 6.4% at T1 (p=0.0454) and 11.0% at T2 (p=0.0049), and CIT-ISO treatment by increasing the latency by 5.3% at T1 (p=0.0188). In addition, CIN-Zn showed significant differences when compared to the other agonists and gum-based TP (Figure 4a).

Regarding the amplitudes, only capsaicin 10µM showed a positive effect on N2-P2 amplitude by 26.1%, although it was not significant (p=0.1563). In contrast, CIN-Zn at T2 significantly decreased it by 36.3% (p=0.0049) and gum-based TP at T1 and T2 by 12.5% (p=0.0132) and 13.7% (p=0.0407), respectively (Figure 4b).

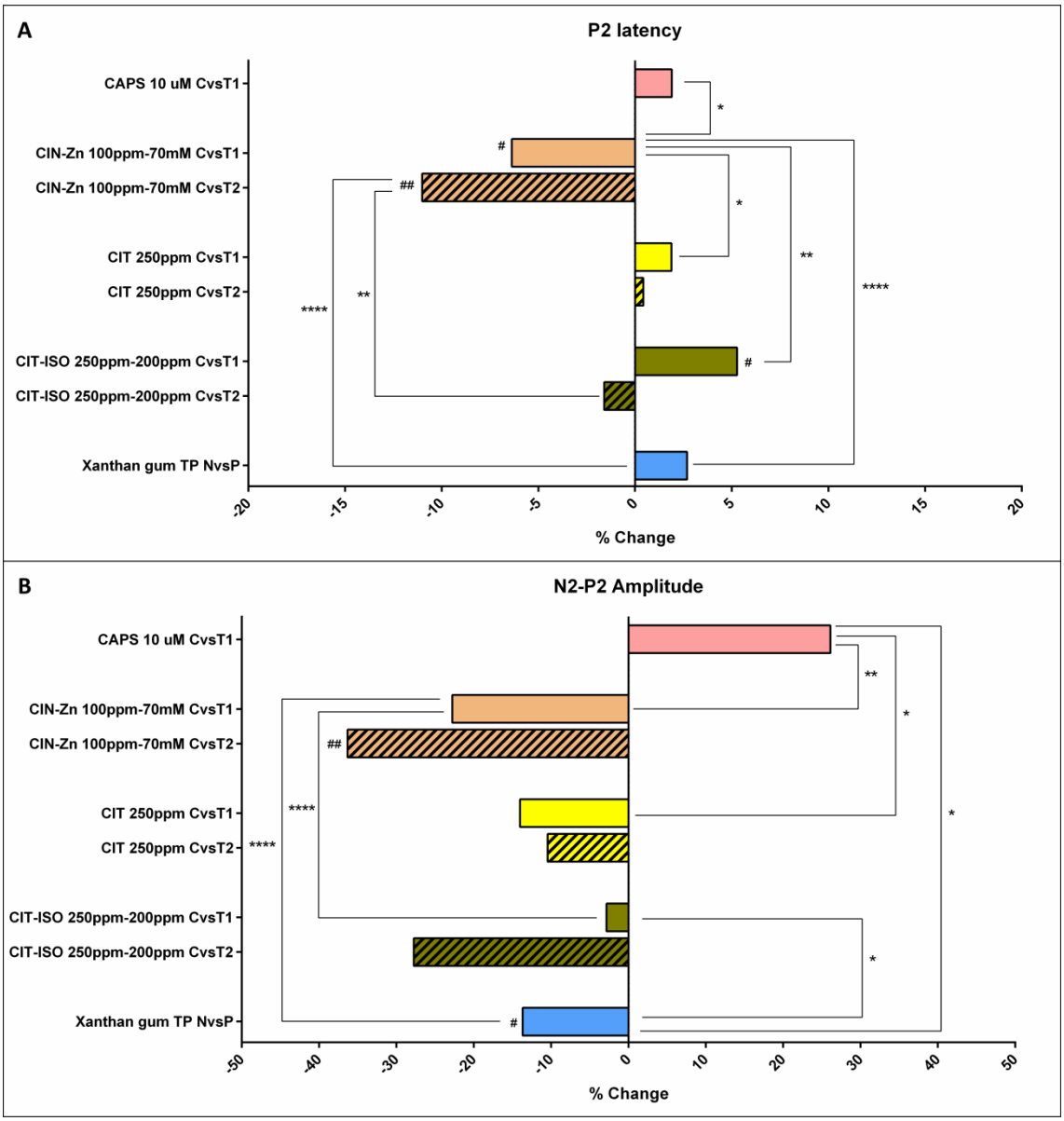

Figure 4: Normalized effect of compensatory and active treatments on the latency of P2 peak (A) and N2-P2 amplitude (B). CAPS: capsaicin; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; C: control nectar; T1: supplemented nectar 1; T2: supplemented nectar 2; N: nectar; P: pudding; TP: thickening product; #: p<0.05; ##: p<0.01 compared to its own control; *: p<0.05; **: p<0.01; ****: p<0.0001 compared to the other treatments.

6. *Effectiveness of pharmacological treatment*

6.1. VFS signs of safety and biomechanics

The one-phase decay curves showed that piperine 1mM was the TRP agonist that reduced the prevalence of signs of safety impairment to a similar extent as TP. However, except for capsaicin 10µM, menthol 10mM and CIT, which were classified in the low therapeutic effect group, most TRP agonists and TPs were classified in the intermediate therapeutic effect group. In addition, piperine 1mM is the pharmacological treatment that had the highest proportion of

patients that improved the prevalence of signs of safety impairment at least 30% (61.1%), significant when compared to menthol 10mM (21.1%, p=0.0201) and CIT (23.8%, p=0.0253) (Figure 5, Table 6).

We also observed in the time to LVC one-phase decay curve that capsaicin 150µM had the highest therapeutic effect on airway protection mechanisms, while piperine 1mM and 150µM, menthol 10mM and 1mM and CIN-Zn had an intermediate effect and capsaicin 10µM, CIT and CIT-ISO had the lowest one (Figure 5, Table 6). The proportion of patients treated with capsaicin 150µM that reduced time to LVC by at least 100ms was 57.6%. This proportion was significantly higher than patients treated with capsaicin 10µM (0.0%, p=0.0089), piperine 1mM (22.2%, p=0.0201), menthol 1mM (25%, p=0.0388), CIT-ISO (18.8%, p=0.0146), starch-based TP, (16.1%, p=0.0008) and gum-based TP (4.65%, p<0.0001). With regard to the effect on time to UESO, capsaicin 150µM, piperine 1mM and 150µM, menthol 10mM and 1mM, CIN-Zn and CIT had an intermediate therapeutic effect, while capsaicin 10µM, CIT-ISO and both TP types had low therapeutic effect. However, no significant differences were found in the proportion of patients whose time to UESO was reduced by at least 100ms (Figure 6).

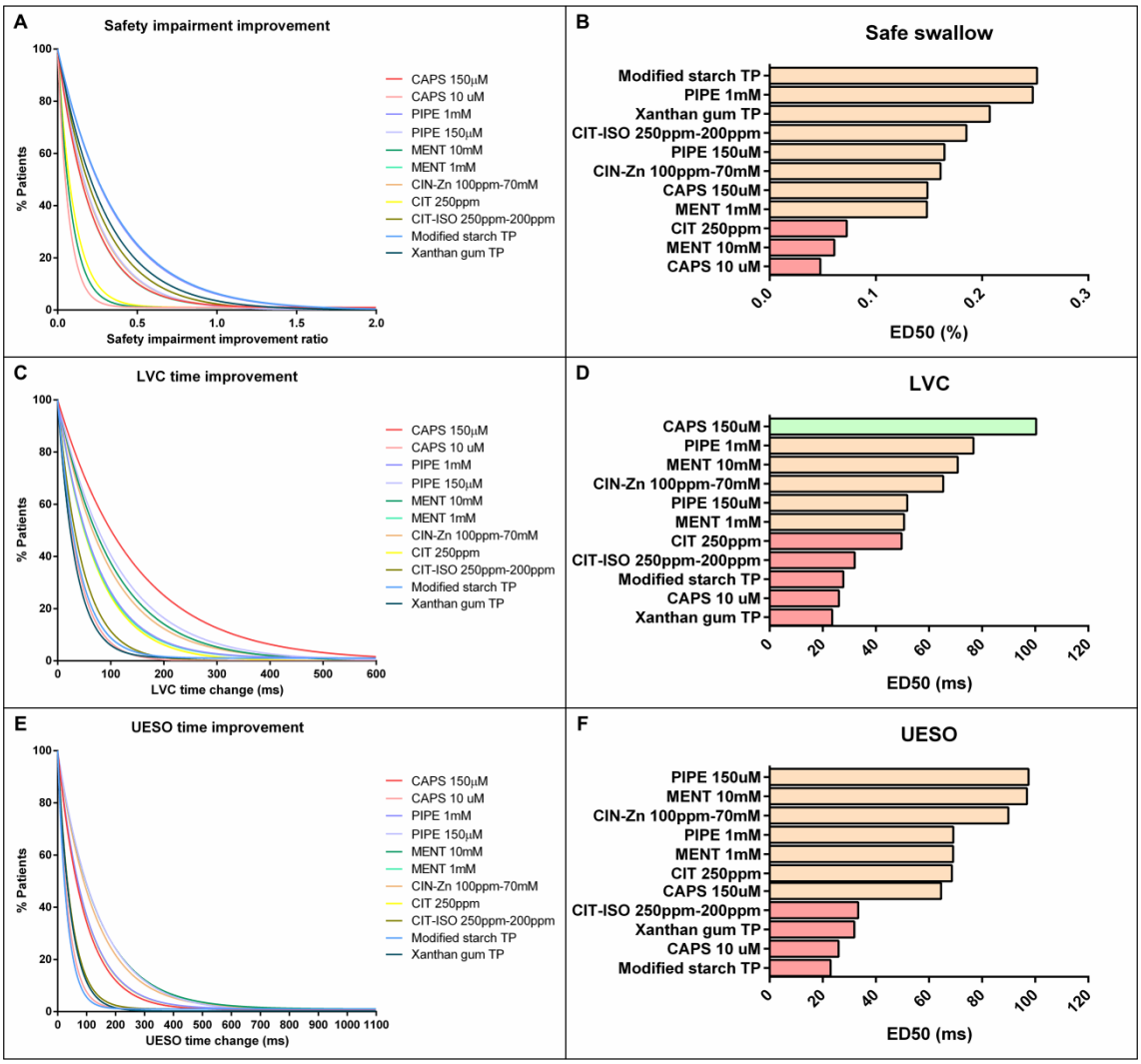

Figure 5: On the left, one-phase decay curves for the proportion of patients with improved signs of safety (A), time to LVC (D) and time to UESO (G). On the right, graphical representation of ED50 of each treatment classified as high (green bars), intermediate (orange bars) and low (red bars) therapeutic effect for signs of safety (B), time to LVC (D) and time to UESO (F). CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; TP: thickening product.

Table 6: Parameters of the one-phase decay curves for the proportion of patients with improved prevalence of safe swallow, time to laryngeal vestibule closure (LVC) and time to upper esophageal sphincter opening (UESO).

		CAPS 150µM	CAPS 10µM	PIPE 1mM	PIPE 150µM	MENT 10mM	MENT 1mM	CIN-Zn	CIT	CIT-ISO	MS TP	XG TP
Safe swallow	**K**	4.728	14.75	2.797	4.213	11.53	4.738	4.335	9.679	3.742	2.753	3.344
	Tau	0.212	0.068	0.358	0.237	0.087	0.211	0.231	0.103	0.267	0.363	0.299
	R^2	0.777	0.455	0.911	0.851	0.525	0.722	0.759	0.549	0.844	0.891	0.270
	ED_{50}	**0.15**	**0.05**	**0.25**	**0.16**	**0.06**	**0.15**	**0.16**	**0.07**	**0.19**	**0.25**	**0.21**
LVC	**K**	0.007	0.027	0.014	0.009	0.010	0.014	0.011	0.014	0.022	0.025	0.041
	Tau	144.8	73.67	110.7	72.00	102.2	93.41	71.71	46.17	37.61	39.50	24.51
	R^2	0.976	0.879	0.908	0.955	0.963	0.804	0.951	0.853	0.920	0.168	0.841
	ED_{50}	**100.37**	**26.07**	**76.73**	**51.83**	**70.83**	**50.63**	**65.37**	**49.72**	**32.00**	**27.78**	**23.58**
UESO	**K**	0.011	0.027	0.010	0.007	0.007	0.010	0.008	0.010	0.021	0.031	0.082
	Tau	92.34	36.92	98.33	140.6	137.8	98.23	127.8	97.57	47.41	32.67	12.2
	R^2	0.891	0.353	0.536	0.845	0.735	0.425	0.676	0.392	0.342	0.119	0.727
	ED_{50}	**64.58**	**25.96**	**69.15**	**97.48**	**96.89**	**69.09**	**89.89**	**68.61**	**33.35**	**22.98**	**31.88**

CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; TP: thickening product; MS: modified starch; XG: xanthan gum.

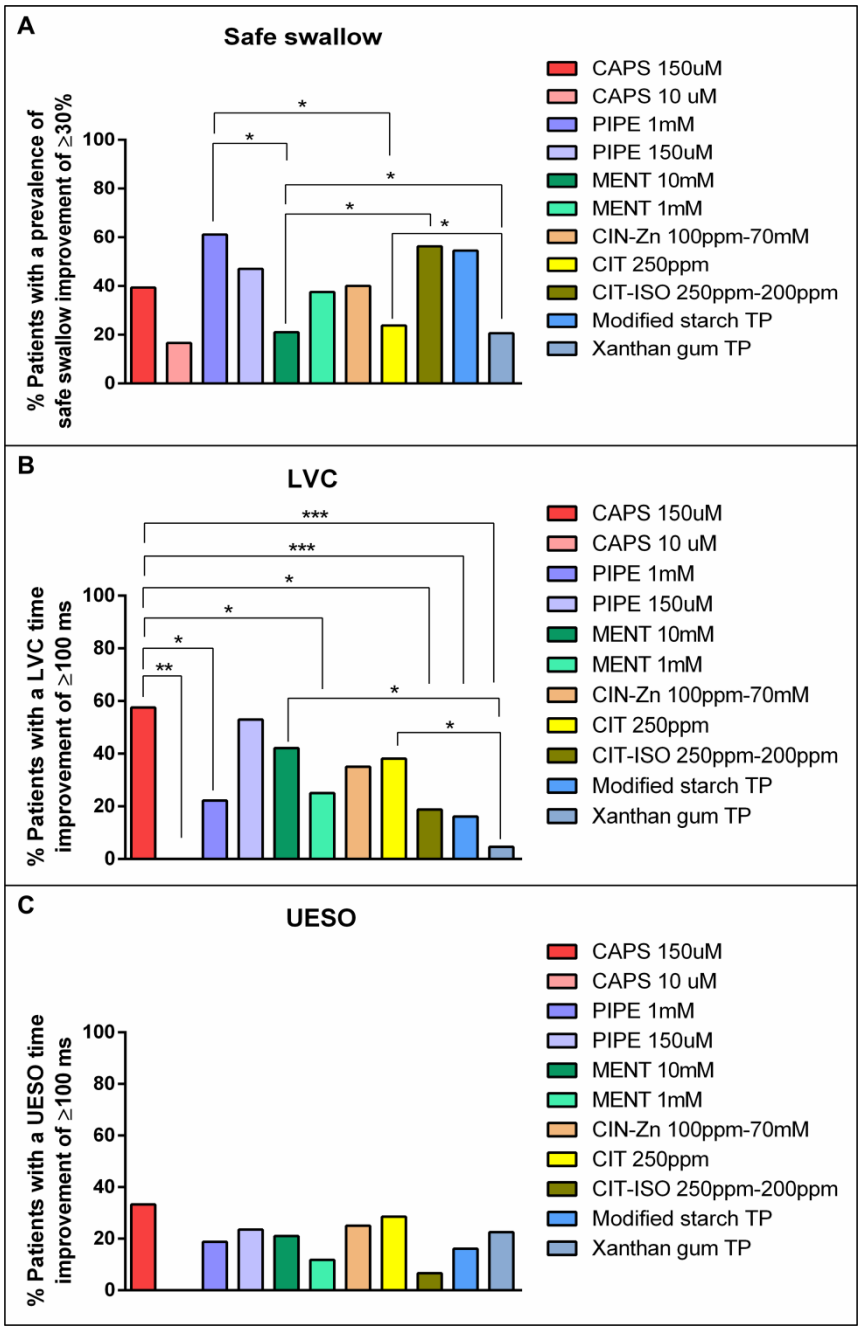

Figure 6: Graphical representation of % of patients that increased safe swallows at least 30% (A), reduced time to of LVC by 100 ms (B) and time to UESO by 100 ms (C). CAPS: capsaicin; PIPE: piperine; MENT: menthol; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; TP: thickening product; *: p<0.05; **: p<0.01; ***: p<0.001.

6.2. Neurophysiology

Regarding the latency of P2 peak, only CIN-Zn was found in the high therapeutic effect group, while capsaicin 10µM, CIT, XG based TP and CIT-ISO were classified as the agents with the lowest effect (Figure 7a,b; Table 7). The proportion of patients that improved the latency of P2 peak after the treatment with CIN-Zn by at least 20ms was 50.0% and only showed significant differences when compared to CIT-ISO (0.0%, p=0.0052) (Figure 8a). Finally, the one-phase

decay curve of N2-P2 amplitude showed that all treatments had a low pharmacological effect (Figure 7c,d; Table 7). No significant differences were found when the proportion of patients that improved by at least 2µV were compared (Figure 8b).

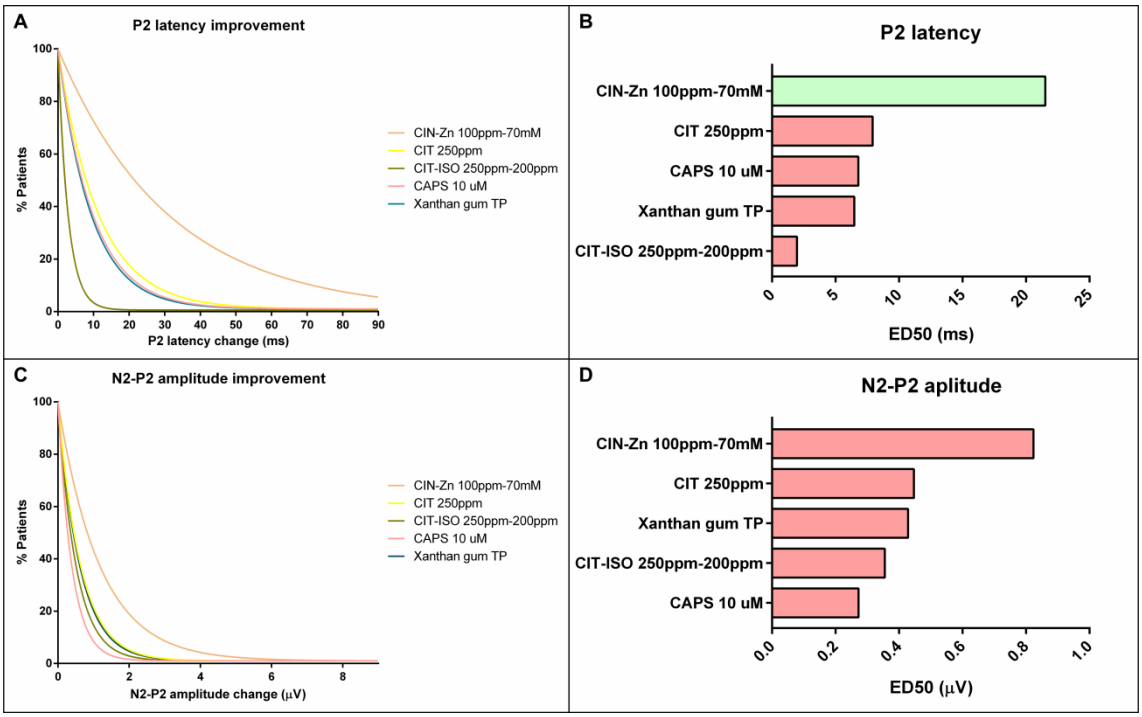

Figure 7: On the left, one-phase decay curves for the proportion of patients with improved P2 peak latency (A) and N2-P2 amplitude (C). In the right, graphical representation of ED50 for each treatment classified as high (green bars) and low (red bars) therapeutic effects for P2 peak latency (B) and N2-P2 amplitude (D). CAPS: capsaicin; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; TP: thickening product.

Table 7: Parameters of the one-phase decay curves for the proportion of patients with improved P2 peak latency and N2-P2 amplitude.

		CAPS 10µM	CIN-Zn	CIT	CIT-ISO	XG TP
P2 latency	K	0.104	0.032	0.089	0.356	0.109
	Tau	9.662	31.05	11.26	2.81	9.211
	R^2	-0.062	0.893	0.356	0.764	0.062
	ED_{50}	**6.79**	**21.52**	**7.92**	**1.96**	**6.48**
N2-P2 amplitude	K	2.582	0.8537	1.574	1.98	1.641
	Tau	0.387	1.171	0.635	0.505	0.609
	R^2	0.467	0.710	0.679	0.801	0.856
	ED_{50}	**0.27**	**0.82**	**0.45**	**0.36**	**0.43**

CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; XG: xanthan gum; TP: thickening product.

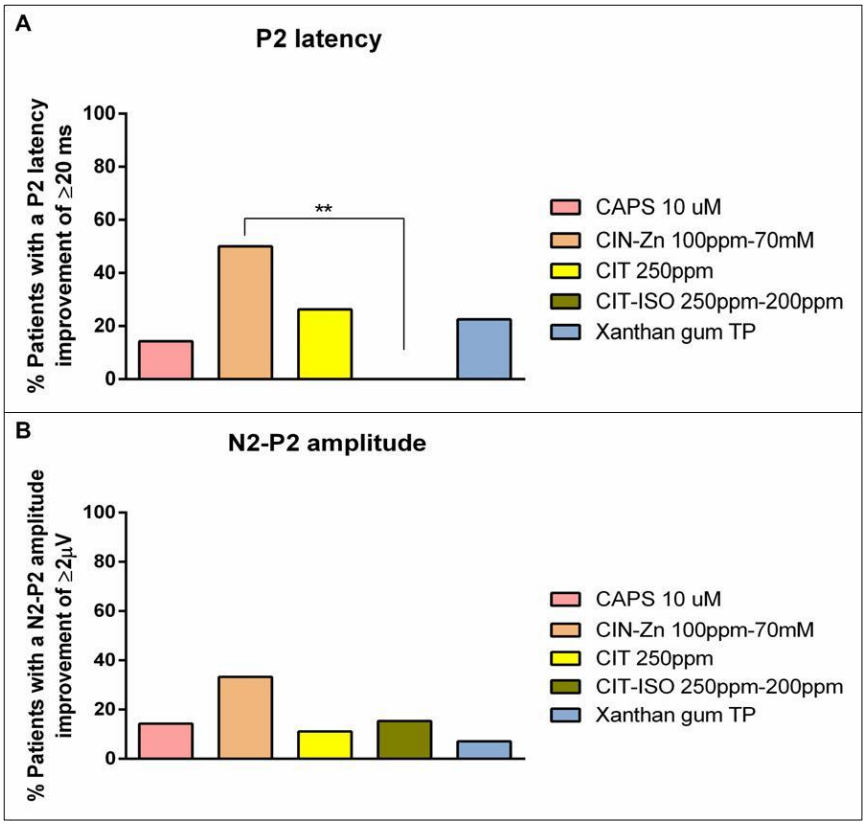

Figure 8: Graphical representation of % of patients that improved P2 peak latency (A) by at least 20 ms and N2-P2 amplitude (B) by 2µV. CAPS: capsaicin; CIN-Zn: cinnamaldehyde-zinc; CIT: citral; CIT-ISO: citral-isopulegol; TP: thickening product. **: $p<0.01$.

Chapter 3: Acute and subacute effects of oropharyngeal sensory stimulation with TRPV1 agonists in older patients with oropharyngeal dysphagia: a biomechanical and neurophysiological randomized pilot study

1. Study dessign

Two study protocols were designed to evaluate the effects of acute and sub-acute treatment with oral capsaicinoids 10μM on the neurophysiology of older OD patients (Figure 1):

a) Acute study: Single dose protocol

Fourteen patients with OD were included in this protocol and randomized to capsaicinoids or placebo intervention. First, socio-demographic and clinical data were collected: age, sex, Barthel index (functional status), Mini Nutritional Assessment short form (MNA-sf) (nutritional status) and Charlson index (comorbidities). All participants were examined with VFS to assess their swallowing function and, after five days, underwent an electroencephalographic study (EEG) to explore the ERP while they received the TRPV1 agonist or placebo.

b) Sub-acute study: Multiple dose protocol

Fourteen OD patients (>70 years) were included in this protocol and randomly allocated to TRPV1 agonist or placebo group. All participants were studied with VFS and EEG before and 5 days after the treatment.

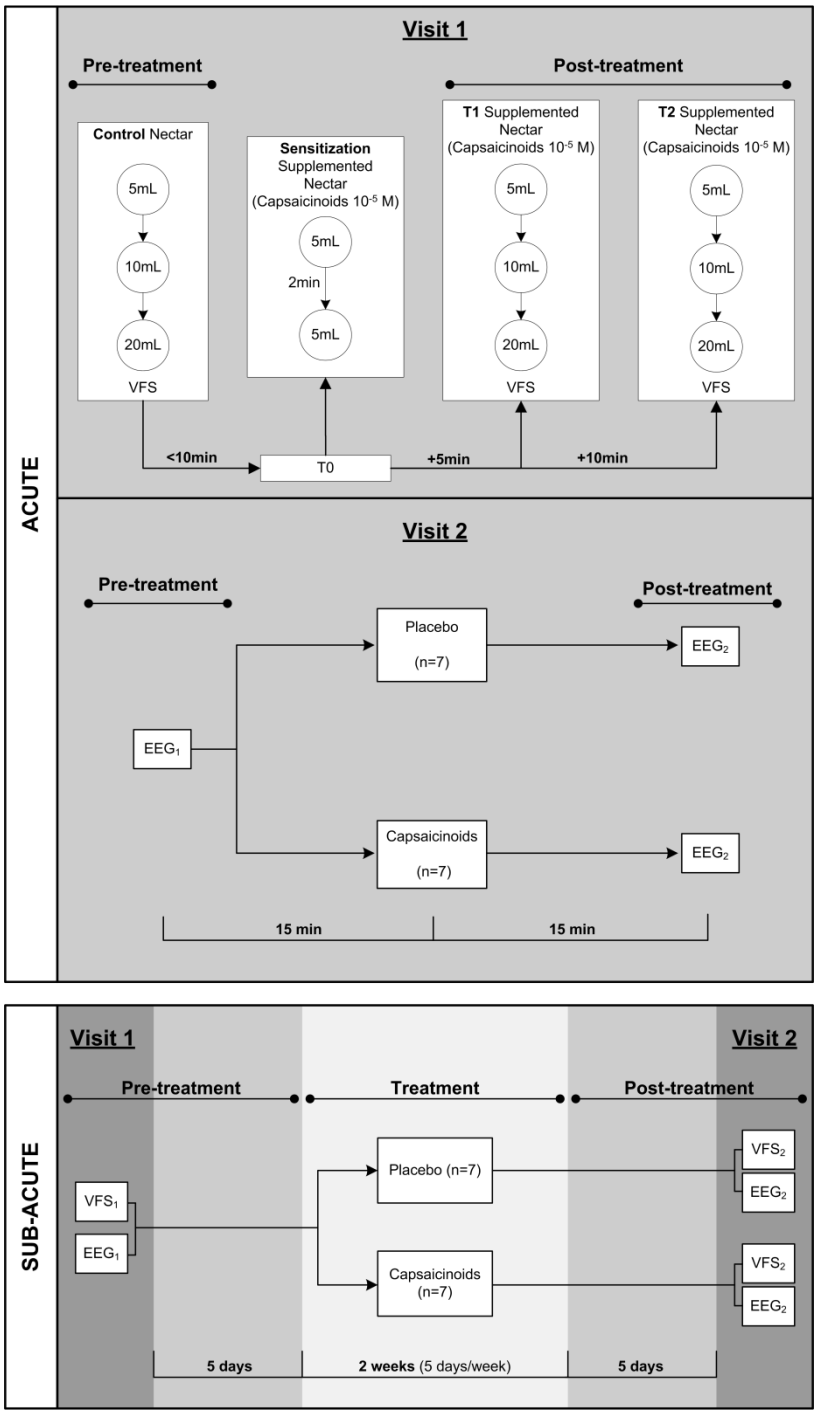

Figure 1: Study design of the acute and sub-acute intervention. VFS, videofluoroscopy; EEG, electroencephalogram; T0, time point 0.

2. *Acute stimulation. Single dose protocol*

2.1. Study population

Fourteen older OD patients (82.9±3.2 years, 9 men) were included in this arm of the study. Patients randomized to active (Group 2) or placebo (Group 1) treatment had similar age, number of comorbidities, functional capacity and nutritional status (Table 1).

Table 1. Socio-demographic and clinical characteristics of the population in the acute and sub-acute arm of the study. Data presented as mean±SD unless specifically stated.

	Acute Study			Sub-acute Study		
	Group 1: Placebo	**Group 2: TRPV1 agonist**	**P-value**	**Group 1: Placebo**	**Group 2: TRPV1 agonist**	**P-value**
N	7	7	1.000	7	7	1.000
Age (years)	83.7±3.9	83.5±6.3	0.530	79.0±5.7	79.8±5.2	0.680
Sex (N, men)	5	4	1.000	5	3	0.550
Barthel index	79.2±25.4	70±33.7	0.567	55±39.1	85.8±16.8	0.200
MNA-sf	12.3±1.9	9.5±2.9	0.106	8.3±3.8	11.7±2.9	0.200
Charlson index	3.7±2.6	3.8±2.5	0.959	2.8±1.9	2.0±1.3	0.570

MNA-sf: Mini nutritional assessment-short form

2.2. Videofluoroscopic results

VFS results showed a population with a high prevalence of VFS signs of impaired efficacy of swallow (mainly pharyngeal residue) and low prevalence of VFS signs of impaired safety of swallow (penetrations) with a moderately delayed timing of OSR. After the single dose treatment with capsaicinoids, patients did not show any significant improvement when compared with the pre-treatment values (Table 2).

Table 2: Swallowing characteristics of the seven patients that received the active single-dose treatment. Data presented as % except for PAS score, LVC and UESO time (mean±SD).

	Pre treatment (n=7)	**Post treatment**		**P-value**
		T1	**T2**	
Impaired Efficacy (%)	85.7	57.1	57.1	0.424
Oral residue (%)	42.9	42.9	42.9	1.000
Pharyngeal residue (%)	85.7	42.9	42.9	0.174
Impaired Safety (%)	28.6	28.6	0.0	0.291
Penetrations (%)	28.6	28.6	0.0	0.291
Aspirations (%)	0.0	0.0	0.0	1.000
PAS score	1.6±1.1	2±1.4	1±0.0	0.296
LVC time (ms)	251.4±72.0	245.7±106.9	211.43±44.5	0.302
UESO time (ms)	228.6±64.1	228.6±72.0	211.4±59.8	0.539

PAS, penetration-aspiration scale; LVC, laryngeal vestibule closure; UESO, upper esophageal sphincter opening.

2.3. Electroencephalographic results

The sensory threshold to electrical stimulation was 13.0±5.4mA for Group 1 and 11.3±4.5mA for Group 2 (P=0.410). The level of intensity at which the stimulus was applied was very similar

between groups (20.6±7.8mA for Group 1 and 22.9±12.0mA for Group 2, P=0.870). Single dose of capsaicinoids 10μM did not produce significant changes in the pSEP (amplitude and latency) (Table 3, Figure 2). We found no correlations between biomechanics and neurophysiology of swallow response in the acute study.

Table 3: Latency and peak-to-peak amplitude of the pharyngeal event-related potential components at Cz electrode following acute stimulation.

	Group 1: Placebo		P-value	Group 2: Capsaicinoids		P-value
	Pre treatment	Post treatment		Pre treatment	Post treatment	
N_1 latency (ms)	89.0±10.6	82.3±17.1	0.534	76.7±19.5	77.0±18.2	0.974
P_1 latency (ms)	143.7±22.7	169.7±40.5	0.106	125.0±11.9	145.3±39.1	0.344
N_2 latency (ms)	241.3±37.8	237.7±55.3	0.870	214.0±46.1	222.3±60.2	0.686
P_2 latency (ms)	353.7±59.6	356.3±77.0	0.955	385.3±77.0	395.0±70.3	0.736
P_1 -N_1 Amplitude (μV)	1.48±1.06	3.87±2.32	0.062	3.74±2.64	4.62±3.30	0.462
N_2 -P_1 Amplitude (μV)	2.29±0.84	1.38±0.88	0.156	3.27±1.63	3.12±1.34	0.687
P_2 -N_2 Amplitude (μV)	2.02±0.89	3.10±1.45	0.156	4.11±1.30	5.19±1.79	0.219

2.4. ERP source localization

In the acute study, no statistical differences were found. In placebo patients, N1 peak distribution showed a weak activation of the occipital lobe; P1 peak showed a bilateral frontal distribution; N2 peak had a bilateral frontotemporal cortical representation; and P2 had a right frontal activation (Figure 2). In the capsaicin group, after the treatment, there was a centralization of the cortical activation in N1 peak; P1 and N1 peaks had an increased cortical representation in the left frontotemporal area and finally, P2 had a moderate increase in the activation of the right frontal lobe.

Using sLORETA, we found the basal anatomical activation in both groups of treatment, showing different localizations according to each one of the peaks from the pharyngeal ERPs: N1 peak activation was found in the middle temporal gyrus (Brodmann area (BA) 21); P1 and P2 in the medial frontal gyrus (BA10); and N2 in the inferior frontal gyrus (BA47).

Compared with basal activation, patients that received the active treatment showed a significant reduction in cortical activity at N1, P1 and N2 peaks (p=0.0002) distributed in the following way: N1 peak showed less activation at the anterior cingulate (BA24); P1 and N2 peaks at paracentral lobule (BA6, premotor cortex); and P2 at cuneus (BA17, primary visual cortex).

3. Sub-acute stimulation. Multiple dose protocol

3.1. Study population

Fourteen additional older OD patients were included in this arm of the study (79.4±5.2 years, 8 men), with similar demographic and clinical characteristics between patients that received the placebo (Group 1) and patients that receive capsaicinoids (Group 2) (Table 1).

3.2. Videofluoroscopic results

Safety signs. All patients showed impaired safety of swallow (PAS>2) before receiving any treatment. After 10 days treatment with capsaicinoids, patients presented a significant reduction in the PAS from 4.14±0.4 to 3.14±0.9 (p=0.038) without changes in the prevalence of aspirations and penetrations. Patients in the control group did not show changes (Table 4). According to the established definition we found a responder rate of 71.43%[219] in Group 2 (capsaicinoids group) and of 28.57% in the Group 1 (control group), but there were no significant differences in responder rate between capsaicinoids and control groups (p=0.2861).

Efficacy signs. We observed alterations in the efficacy of 100% of the participants. Neither the patients treated with capsaicinoids nor the control group presented changes after treatment (Table 4).

Oropharyngeal swallow response. Patients in the control group (Group 1) did not present changes in the timing of the OSR. In contrast, patients treated with capsaicinoids (Group 2), showed a statistically significant reduction in the LVC time from 457.3±46.8ms to 354.3±53.8ms (p=0.042) and the UESO time from 348.6±88.6ms to 285.7±78.66ms (p=0.125) (Table 4).

3.3. Electroencephalographic results

The sensory threshold of patients included in Group 1 and 2 was 11.0±3.1mA and 7.0±5.4mA respectively (p=0.173). The intensity level at which the stimulus was applied did not change between sessions: Group 1 received a stimulation intensity of 24.5±8.1mA before treatment and 24.2±8.5mA after treatment (p=1.00) and Group 2 received a stimulation of 16.08±5.1mA before treatment and 15.7±5.6mA after treatment (p=0.872).

Two-week treatment with capsaicinoids induced significant changes in the pharyngeal ERP: a reduction in the latency of the N1 peak (from 90.0±18.6ms to 64.3±16.8ms, p=0.007) and an increase in the amplitude of the P1-N2 (from 1.3±1.4μV to 3.2±1.9μV, p=0.038) and N2-P2 (from 3.1±1.0μV to 5.5±2.3μV, p=0.050) peaks (Figure 2, Table 4). In contrast, patients in the

control group did not show any change in their pSEPs or their cortical activation after the two-week treatment (Figure 2, Table 4). Pre-treatment and post-treatment scalp distributions of the pharyngeal ERP showed no statistically significant differences in the placebo group.

Table 4. Swallowing characteristics and latency and peak-to-peak amplitude of the pharyngeal event-related potential components at Cz electrode of the placebo and capsaicinoids group.

	Group 1: Placebo		**P-value**	**Group 2: Capsaicinoids**		**P-value**
	Pre treatment N=6	**Post treatment N=6**		**Pre treatment N=6**	**Post treatment N=6**	
Swallowing characteristics (VFS)						
Impaired Efficacy (%)	100	100	1.000	100	83.3	1.000
Oral residue (%)	100	100	1.000	83.3	66.7	1.000
Pharyngeal residue (%)	83.3	66.7	1.000	83.3	83.3	1.000
Impaired Safety (%)	100	100	1.000	100	83.3	1.000
Penetrations (%)	66.7	100	1.000	100	83.3	1.000
Aspirations (%)	33.3	16.7	0.455	0	0	1.000
PAS Score	5.0±1.3	5.17±1.0	0.741	4.17±0.4	3.33±0.8	0.092
LVC time (ms)	360.0±147.5	406.7±81.7	0.527	453.3±112.2	366.7±46.8	0.136
UESO time (ms)	300.0±65.7	300.0±49.0	1.000	353.3±73.4	293.3±78.7	0.097
Pharyngeal ERP (EEG)						
N_1 latency (ms)	81.7±12.2	78.7±16.3	0.634	93.3±17.4	66.3±17.4	**0.015**
P_1 latency (ms)	146±31.2	150.7±28.8	0.371	124.7±20.4	119±18.4	0.673
N_2 latency (ms)	225.7±31.1	208.3±29.1	0.262	239±70.0	261.7±47.7	0.165
P_2 latency (ms)	332±26.4	319.3±49.6	0.313	372.7±77.6	409.7±38.6	0.124
P_1-N_1 Amplitude (μV)	3.4±1.3	3.8±1.2	0.176	1.8±1.4	3.3±1.8	0.089
N_2 -P_1 Amplitude (μV)	0.7±1.0	1.8±1.8	0.227	1.2±1.5	3.7±1.5	**0.001**
P_2 -N_2 Amplitude (μV)	3.9±3.9	4.0±3.5	0.842	2.9±1.1	6.1±1.7	**0.010**

PAS, penetration-aspiration scale; LVC, laryngeal vestibule closure; UESO, upper esophageal sphincter opening.

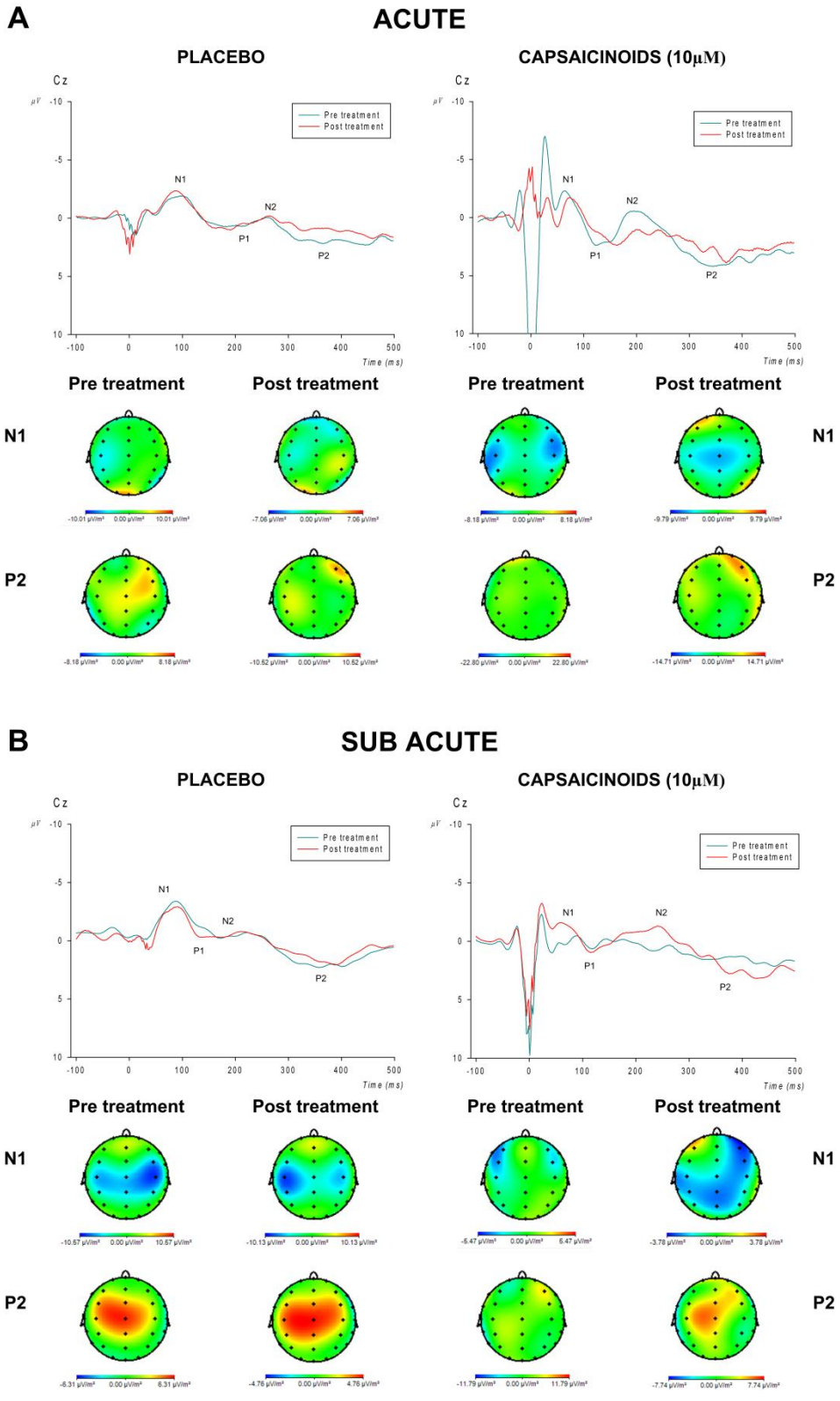

Figure 2: Pharyngeal event-related potential and scalp density maps to pharyngeal ERPs for acute (A) and sub-acute (B) studies. At the top of each treatment, event-related potential (ERP) traces obtained at Cz electrode for pre-treatment (blue line) and post-treatment (red line) from placebo group and capsaicinoids group after pharyngeal electrical stimulation. Deflection at time point 0 corresponds to stimulus artifact. At the bottom, current scalp density maps at each ERP peak time point for each group.

4. Correlation between VFS and EEG results

The reduction observed in the latency of the N1 component of the pharyngeal ERP after the sub-acute treatment strongly and significantly correlated (r=0.750; p=0.003) with the reduction observed in the LVC time, the main airway protection mechanism during swallow. In contrast there was no correlation when we analyzed the reduction observed in the latency of the same peak with that of the overall duration of the swallow response until the UES closure time (r=-0.06026; p=0.7503) (Figure 3).

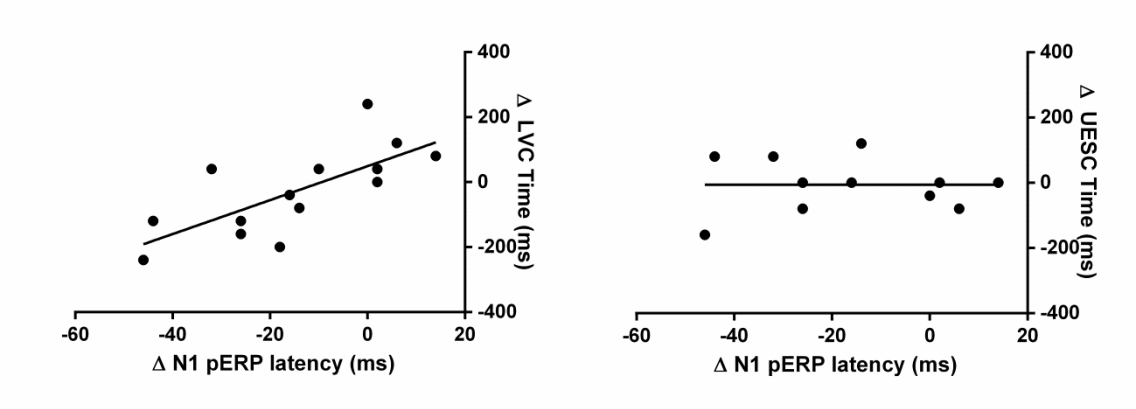

Figure 3. Correlation between the change in LVC time (left) and UESC time (right) and the reduction of the latency of N1 peak after the multiple dose treatment. LVC: laryngeal vestibule closure; UESC: upper esophageal sphincter closure; pERP: pharyngeal event related potential.

5. ERP source localization

Patients that received the placebo in the sub-acute study did not show changes in cortical activation distribution. N1 peak had a bilateral cortical representation; P1 peak had a frontoparietal and left frontal lobe representation; N2 peak had a right temporal and frontoparietal distribution; and P2 peak had a wide centroparietal representation (Figure 2). After two weeks treatment with capsaicinoids, we also found statistically significant differences (Table 4). N1 cortical activation changed from bilateral frontal distribution to a frontoparietal and temporal distribution; P1 cortical representation (not shown) changed from a unilateral frontal representation to a more bilateral frontal distribution; N2 temporal bilateral activity was reduced and P2 activity was strongly increased with higher representation in frontoparietal lobes (Figure 2).

The basal anatomical activation was similar to that described in the acute study. The localization of each peak was: BA21 in N1 peak, BA10 in P1 and P2 peak, and BA47 in N2 peak. After two weeks of treatment with capsaicinoids, we observed an increase in the cortical activation at N1, N2 and P2 peaks ($p<0.0001$) represented as increased activity in N1 and P2

peaks on the cingulate gyrus (BA31); in P1 at paracentral lobule (BA5, somatosensory association cortex); and N2 at the medial frontal gyrus (BA8 secondary motor cortex) (Figure 4).

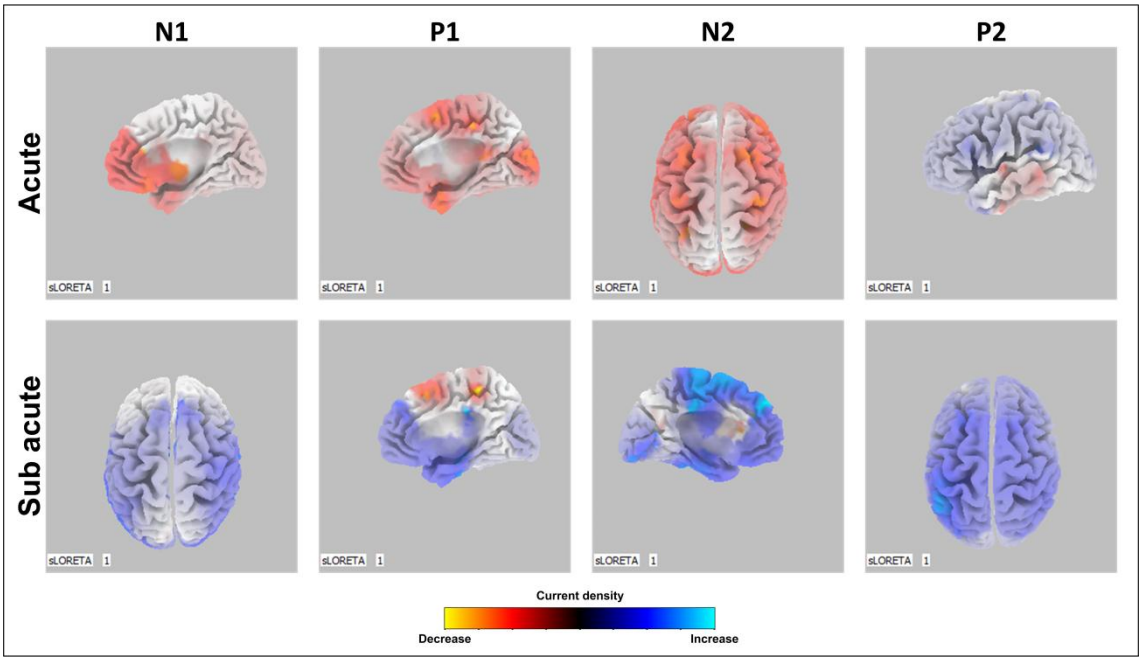

Figure 4. Differences in LORETA source activity after acute stimulation (top) and sub-acute stimulation (bottom) compared with basal cortical activity. Colored voxels represent areas of significant differences in activation (blue, increase; red, decrease) after correction for multiple comparisons.

6. Safety of the Treatment

During the study there were no adverse or serious adverse events. Thus, we concluded that our treatments were safe for our older patients with OD.

Chapter 4: Oropharyngeal dysphagia in older people is associated with reduced pharyngeal sensitivity and low substance P and CGRP concentration in saliva

1. Study design

The study was conducted in the Gastrointestinal Motility Laboratory of the Hospital de Mataró and consisted of only one visit. Healthy elderly and elderly with OD were screened with the V-VST to determine clinical signs of OD and assessed by VFS. Those with a Penetration-Aspiration Scale (PAS) score higher than 2 during VFS were classified as elderly with OD. Socio-demographic and clinical data (functional status: Barthel index; nutritional status: Mini Nutritional Assessment short form (MNA-sf); and comorbidities: Charlson index) and a sample of saliva were collected from all participants. A bioimpedance study was also performed to assess body composition and hydration status of study individuals. Finally, the pharyngeal sensory threshold to intrapharyngeal electrical stimulation was determined in each participant by triplicate.

2. Study population

The study included 43 participants divided into three groups with a similar distribution of men and women. Most patients in the healthy elderly and elderly with OD groups presented a preserved functional status, were well nourished and had a low number of comorbidities, without significant differences between groups (Table 1).

Table 1. Socio-demographic and clinical data of the HV, HE and EOD groups. Data expressed as mean±SD.

	HV	HE	EOD
n	15	14	14
Sex (men:women)	7:8	5:9	7:7
Age (years±SD)	32.73±8.34	74.36±6.49****	79.14±6.37****
Barthel index	100	95.00±8.99	97.86±6.71
MNA-sf	14	13.14±1.35	12.57±1.79
Charlson index	0	1.50±1.22	1.07±0.62

HV: healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia; MNA-sf: Mini Nutritional Assessment short form; ****: p<0.0001 compared with HV.

3. VFS assessment of swallow function

All the study participants were assessed with VFS. Healthy volunteers showed no signs of impaired safety and/or efficacy, with a mean time to LVC and to UESO of 188.00±45.23 ms and 157.33±43.99 ms respectively. Up to 78.57% of healthy elderly had mild efficacy impairments

(oral and/or pharyngeal residue), with a mean PAS score of 1.43±0.51 and with preserved mean time to LVC and to UESO (221.82±60.30 ms and 218.18±45.13 ms, respectively). In contrast, all patients classified as elderly with OD presented signs of both impaired efficacy and safety of swallow and a mean PAS score of 4.38±0.77. In addition, elderly patients with OD presented a significant delay in the time to LVC (346.67±78.78 ms, p=0.0004) and to UESO (276.67±77.14 ms, p=0.024) when compared to healthy elderly (Table 2).

Table 2: HV, HE and EOD VFS results. Data expressed as mean±SD.

	HV	HE	EOD
Efficacy impairment (%)	0	78.57****	100****
Oral Residue (%)	0	57.14***	64.29***
Pharyngeal Residue (%)	0	57.14***	78.57****
Safety impairment (%)	0	0	100****;####
Penetrations (%)	0	0	100****;####
Aspirations (%)	0	0	7.14
PAS score	1	1.43±0.51	4.38±0.77****;####
Time to LVC (ms)	188.00±45.23	221.82±60.30*	346.67±78.78****;###
Time to UESO (ms)	157.33±43.99	218.18±45.13	276.67±77.14***

HV: Healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia; PAS: Penetration-Aspiration Scale; LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening; ****: p<0.0001 compared to HV; ***: p<0.001 compared to HV; *: p<0.05 compared to HV; ####: p<0.0001 compared to HE; ###: p<0.001 compared to HE; #: p<0.05 compared to HE.

4. Body composition, hydration status and saliva volume

Bioimpedance measurements showed that healthy elderly and elderly with OD presented significant changes in body composition, mainly an increased fat mass and a reduced lean mass and muscular mass vs healthy volunteers. Elderly with OD also presented a reduction in the cellular mass (Table 3). Both healthy elderly and elderly with OD showed significant hypertonic dehydration as the relationship between the extracellular water and the intracellular water was significantly increased in healthy elderly (1.36±0.38, p=0.0014) and elderly with OD (2.31±1.35, p=0.0002) and the phase angle was significantly reduced (healthy elderly: 3.79±1.40º, p=0.0049; elderly with OD: 2.77±1.86º, p=0.0017) when compared with healthy volunteers (extracellular water/intracellular water: 0.66±0.22; phase angle: 6.55±2.30º) as a consequence of the reduction in intracellular water (Figure 1a). In addition, the volume of the saliva sample collected was smaller in healthy elderly and elderly with OD (healthy volunteers: 1333.33±615.91µl vs healthy elderly: 592.86±327.79µl, p=0.0004; elderly with OD: 422.00±343.01µl, p=0.0001) (Figure 1b) showing a strong positive correlation between saliva volume and hydration status assessed by phase angle (r=0.6621, p<0.0001) (Figure 1c).

Table 3: Bioimpedance and body composition. All data are expressed as mean±SD.

	HV	HE	EOD
Total water (%)	52.41±7.31	51.22±17.86	50.51±7.24
ECW (%)	42.15±13.83	56.76±6.17**	65.63±12.15***
ICW (%)	57.85±13.83	43.24±6.17**	34.22±12.07***
ECW/ICW	0.66±0.22	1.36±0.38**	2.31±1.35***
PA (º)	6.55±2.30	3.79±1.40**	2.77±1.86**
Na/K	0.85±0.27	1.09±0.39	0.98±0.54
Cellular mass (%)	54.58±12.38	46.21±17.10	33.48±11.62**
Fat mass (%)	25.63±7.49	41.91±6.63****	36.83±9.05**
Lean mass (%)	74.37±7.49	58.09±6.63****	63.18±9.05**
Muscular mass (%)	51.69±2.50	34.27±9.86**	28.72±8.14***
Basal metabolism (Kcal)	1599.49±169.04	1295.04±176.67*	1261.72±174.38*

HV: Healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia; ECW: extracellular water; ICW: intracellular water; PA: phase angle; ****: p<0.0001 compared to HV; ***: p<0.001 compared to HV; **: p<0.01 compared to HV; *: p<0.05 compared to HV.

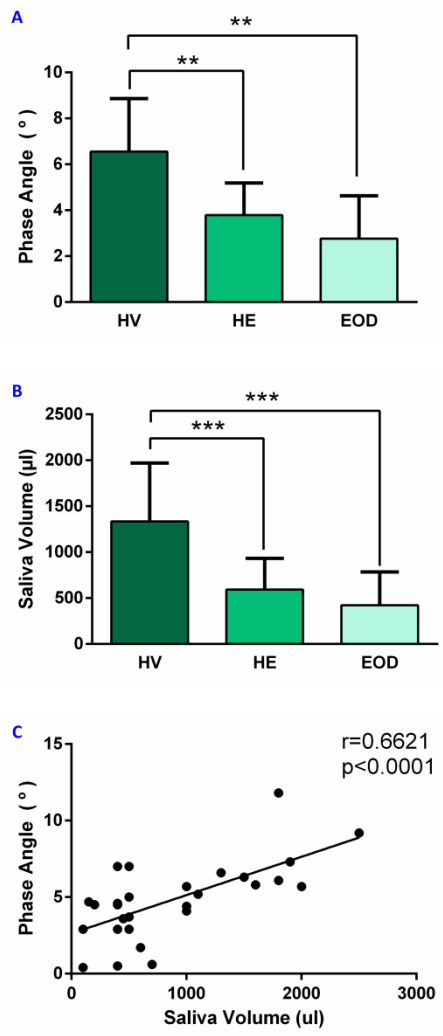

Figure 1: Bioimpedance results and saliva volume in the three groups: a) Phase angle; b) Saliva volume; c) Correlation between phase angle and saliva volume. HV: healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia. º: degree; µl: microliter; **: p<0.01; ***: p<0.001.

5. Pharyngeal sensory threshold

The pharyngeal sensory threshold in healthy volunteers was 5.75±2.48 mA, which was poorer in healthy elderly (7.57±2.53 mA, p=0.6897) and significantly impaired in elderly with OD (10.80±3.72 mA, p=0.007), clearly showing a reduced pharyngeal sensitivity in elderly patients with OD. Up to 14.29% of healthy elderly and up to 35.71% of elderly with OD had pharyngeal sensory threshold above the upper limit of the reference interval (10.71 mA).

6. SP and CGRP concentrations in saliva and its correlation with pharyngeal sensitivity

Regarding the salivary concentration of the neuropeptides, healthy elderly (168.36±109.26 pg/ml) and elderly with OD (139.96±47.42 pg/ml) showed a non-significative decrease in SP when compared with healthy volunteers (192.34±116.57 pg/ml) (Figure 2a), while the reduction of CGRP was significant in both older groups (healthy elderly: 45.07±40.43 pg/ml, p=0.0089; elderly with OD: 24.17±6.99 pg/ml, p=0.0058; vs healthy volunteers: 508.18±286.29 pg/ml) (Figure 2c).

There was a moderate negative correlation between the pharyngeal sensory threshold and salivary SP concentration (r=-0.450, p=0.024) and CGRP (r=-0.4597, p=0.036) (Figure 2b and d).

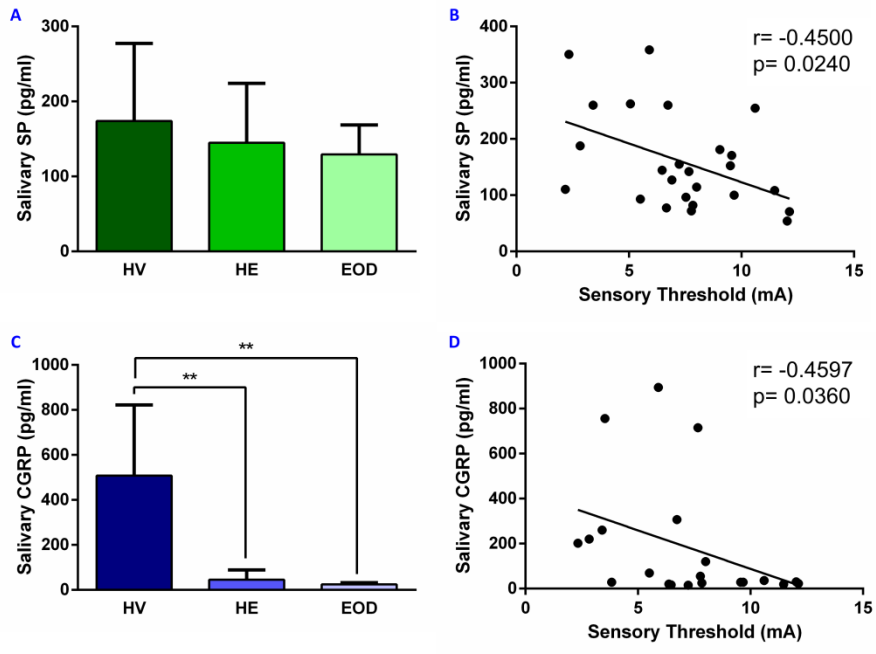

Figure 2: Salivary neuropeptides and their correlation with pharyngeal sensory threshold. a) Salivary SP concentration; b) Correlation between pharyngeal sensory threshold and salivary SP; c) Salivary CGRP concentration; d) Correlation between pharyngeal sensory threshold and salivary CGRP. HV: healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia; mA: milliampere; pg/ml: picogram/milliliter; **: p<0.01.

In addition, the pharyngeal sensory threshold was higher in those healthy volunteers and elderly with OD participants with the lowest salivary SP levels (healthy volunteers: 7.80±1.36 mA; elderly with OD: 8.97±2.35 mA) than in those with higher SP levels (healthy volunteers: 3.94±1.36 mA, p=0.0047; elderly with OD: 5.19±1.39 mA, p=0.049) (Figure 3a). When considering both older groups together (healthy elderly and elderly with OD), pharyngeal sensory threshold was also higher in those with reduced concentration of SP in saliva. (Figure 3a). In contrast, the differences between the pharyngeal sensory threshold in patients with low CGRP concentration compared to those with high CGRP did not reach statistical significance when analyzed by group or when considering all both older groups together (Figure 3b).

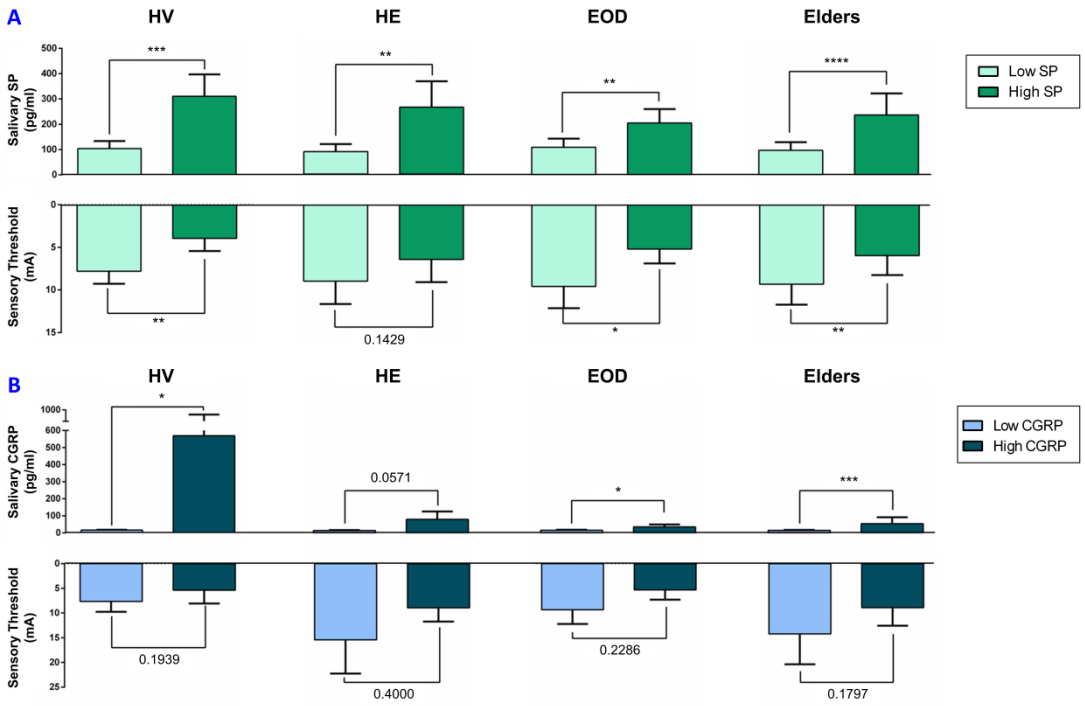

Figure 3: Division of the groups according to salivary SP and CGRP concentration. a) Concentration of salivary SP and pharyngeal sensory threshold in low and high SP groups; b) Concentration of salivary SP and pharyngeal sensory threshold in low and high CGRP groups. HV: healthy volunteers; HE: elders without oropharyngeal dysphagia; EOD: elders with oropharyngeal dysphagia; pg/ml: picogram/milliliter; mA: milliampere;*: p<0.05; **: p<0.01; ***: p<0.001.

DISCUSSION PER CHAPTER

Chapter 1.1: A randomized clinical trial on the acute therapeutic effect of TRPA1 and TRPM8 agonists in patients with oropharyngeal dysphagia

The main results of this study are that supplementation of the nectar bolus with cinnamaldehyde and zinc (CIN-Zn, TRPA1 agonists) significantly improved swallowing safety and the neurophysiology and biomechanics of the swallow response in our patients. The study also shows that CIN-Zn is safe, palatable and well tolerated. On the other hand, citral (TRPA1 agonist) and the combination of citral and isopulegol (TRPA1/M8 agonists) slightly improved the swallow response but had no significant effect on the VFS signs of safety impairments of OD. These results suggest the benefits of using the TRPA1 agonist CIN-Zn (756.6µM cinnamaldehyde and 70µM zinc) as an active treatment for OD when combined with a xanthan gum thickener at 182±2mPas·s viscosity.

Prevalence of malnutrition, co-morbidities and polypharmacy with potential effects on swallowing function among the study populations was very high, putting patients at risk of developing further serious respiratory –aspiration pneumonia- and nutritional complications, readmissions and mortality [94], [139], [146]. Our restrictive inclusion criteria was designed to homogenize the sample as much as possible to investigate the immediate effect of TRP agonists on swallow biomechanics and neurophysiology in patients receiving thickening agents as compensatory treatment. The high dropout rate observed after the VFS in our study was caused by the inclusion criteria which required a positive V-VST during the deglutition of any volume and viscosity level whereas the randomization criteria was to have a PAS>2 during the 5 ml nectar bolus in the VFS study. This study further confirms the severe impairment of the biomechanics of the OSR and pharyngeal sensory function in patients with OD associated with aging and neurological diseases. VFS signs of impaired safety were related to a delayed swallow response, more precisely to a delayed time to laryngeal vestibule closure (LVC), as found in our previous studies [119], [133].

Time to LVC is a critical biomechanical event, relevant to the occurrence of penetrations and aspirations into the airway [119], [133]. The results of this study and our previous studies suggest that reducing time to LVC and improving bolus propulsion and velocity are key targets for active treatments aiming at improving the swallow response in patients with OD [133].

OD was also associated with impaired oropharyngeal sensory perception in this study. In previous studies of our group, we found that older HV and older OD patients had significantly higher sensory thresholds than young HV [86]. The decrease of pharyngeal sensitivity with age had been also described by Aviv et al. [249] and related to neurodegenerative peripheral nerve impairments [250]. The patients included in this study also had a high pharyngeal sensory threshold to electrical stimuli and a longer N2 latency and lower N1-P1 and N2-P2 amplitudes than HV, agreeing with our previous studies [86]. The sLORETA analysis showed that the cortical source localization of sensory perception in our patients was mainly focalized in areas of the frontal lobe and the cingulate cortex.

In this study, the mean PAS score at nectar viscosity was almost 4 and CIN-Zn reduced PAS from 4.00±1.48 at T0 with the nectar control series to 3.19±1.81. Nectar supplemented with any of the treatments assayed in this study improved the OSR timing, but only CIN-Zn significantly improved swallow safety. The stimulation of TRPA1 with CIN-Zn significantly reduced the PAS score and the prevalence of laryngeal penetrations by significantly shortening time to LVC, similar to the effect we found with piperine, an agonist that activates both TRPV1 and TRPA1, in an earlier study [224], [226]. Furthermore, CIN-Zn significantly reduced the time to UESO, an effect we found with capsaicinoids [218]. In our study, CIT (TRPA1 agonist) improved both the time to LVC and to UESO, and prevalence of safe swallows but in contrast CIT-ISO (TRPA1/TRPM8 agonists) only had a significant effect on the time to LVC. A cross-desensitization effect between cinnamaldehyde (TRPA1 agonist) and menthol (TRPM8 agonist) has been described in rat trigeminal subnucleus caudalis neurons and could be the cause of the difference between the effect of our TRPM8 and TRPA1 agonists [251]. In addition, we observed significant neurophysiological improvements with patients who received CIN-Zn treatment who showed a shorter P2 peak latency in comparison with placebo, after the first (-23.8ms) and the second (-41.2ms) administration, and a reduction in the N2-P2 amplitude in the pSEP2 (10.66±9.64% of change, p=0.049). These neurophysiologic improvements could be related to the biomechanical improvements observed and these effects on brain plasticity should be taken into account in future studies.

The TRP agonist concentrations assayed in this study were similar to those used in flavorings and were perceived as pleasant and were well tolerated by OD patients who did not show signs of gut discomfort. Only the CIT IP was perceived as significantly more intense and less pleasant compared to placebo. Finally, studies from our group using a TRPV1 agonist (capsaicinoids) therapy over ten days showed improvements in both biomechanical and neurophysiological swallowing responses without any desensitization processes [246], [252].

This suggests that chronic treatments with TRP agonists could be useful as an active treatment for OD but more studies are needed to evaluate the long-term effect of this promising therapy.

This study has some limitations. The first one is that we included three phenotypes of patients with OD in the study (older, neurodegenerative disease, stroke), further studies with larger sample sizes of each phenotype are needed in order to make sub-analyses comparing the effect of the treatment between phenotypes. In addition we evaluated the acute effect (single dose) of the TRPA1 and TRPM8 agonists. Studies describing the long term effect of repetitive doses of these agonists will clarify whether the observed biomechanical and neurophysiological changes are maintained over time. Finally, whereas the EEG studies had a placebo arm in a crossover design, the VFS studies did not have a separate control group to avoid unnecessary exposure to radiation. However, the improvements observed in biomechanics after treatment with the TRP agonists cannot be explained by learning effect although the order of exposition was always the same. We did not find any improvement at T0 when comparing the boluses of 5, 10 and 20 ml during VFS, allowing us to attribute the improvement to the TRP agonists.

In summary, TRPA1 acute stimulation with CIN-Zn or CIT improves the swallow response which, in the case of CIN-Zn, is associated with a significant modification in cortical activation and improves swallowing safety. These results are foundational for the development of new active pharmacological treatments for OD by combining TRPA1 agonists and a xanthan gum thickener. This is a relevant advance in the new paradigm of treatments for patients with oropharyngeal dysphagia, from compensation to the recovery of swallow function.

Chapter 1.2: Effect of aging, gender and sensory stimulation of TRPV1 receptors with capsaicin on spontaneous swallowing frequency in patients with oropharyngeal dysphagia: a proof-of-concept study

The aims of this proof-of-concept study were to describe the effect of age and gender on SSF and its associated metrics, to assess whether patients with post-stroke OD had impaired SSF and to assess the potential therapeutic effect of oropharyngeal sensory stimulation with a TRPV1 agonist such as capsaicin on SSF. In addition, we also explored the behaviour of some metrics associated with SSF (amplitude, duration, AUC) in both experimental situations. We found SFF was significantly reduced by age but not by gender in HV. We also observed a trend towards reduced amplitude and duration of SSF with age. Interestingly, post-stroke dysphagic patients showed increased basal amplitude, duration and AUC when compared to older healthy people. Acute stimulation with capsaicin caused a

significant double-fold increase in SSF, further suggesting the potential role of sensory stimulation as a therapeutic strategy for CPG activation in dysphagic patients without effect on amplitude or duration.

The first result of the study was a significant reduction of SSF with age, a 29.13% reduction in GII group (40-59 years old) and 51.47% in GIII group (>60 years old) in comparison to GI group (18-39 years old). We also observed that there was a significant negative correlation between age and SSF. These results concur with those previously described [253], [254]. In addition, there was a reduction in the duration of each swallow in the GIII group compared to GI. Spontaneous swallowing is a rhythmic motor behaviour, such as breathing and sucking [255] and its motor control depends on both the peripheral and central nervous systems. Although there is no evidence on how aging impairs spontaneous swallowing, in previous studies we found that older people show a decrease in pharyngeal sensitivity and significant alteration of the pharyngeal sensory evoked potentials, much more pronounced in older patients with OD [86]. This impairment in the sensory function is related to a reduction in the small myelinated fibers of the superior laryngeal nerve [250] and peripheral neurodegeneration of the oropharyngeal mechanoreceptors fibers, alterations that correlate with impaired mastication and swallowing function in an aging animal model [256]. We previously found that this impairment in the afferent pathway of the neurophysiological swallowing response was associated with the impaired biomechanics of the oropharyngeal swallow response (OSR). Several studies from our group have shown that older people, especially those with OD, present an altered OSR, especially delayed time to LVC, which leads to an increase in the prevalence of penetrations and aspirations [119]. In this study we found a similar effect of age on the reduction of SSF paralleling the decrease in pharyngeal sensory function and the delay in time to LVC. When we analyzed the data by age and gender, we observed that women showed a significant reduction only in SSF in GIII in comparison with the GI group. On the other hand, men showed a significant reduction in both GII and GIII groups when compared to the GI group. When we performed a multiple linear regression analysis, however, we found that SSF was only affected by the variable age but not by gender.

Regarding the EMG metrics, the amplitude, delta T and AUC of each swallow were evaluated. The amplitude represents the maximal force contraction; the delta T, the duration of the muscular contraction, and the AUC, the integral of the amplitude and the delta T of the suprahyoid muscle contraction showing the overall muscular effort during each swallow. We observed a reduction in the amplitude in women with age while in men it was the duration of each swallow that was affected by age. These results agree with what has been previously

described [257]–[259] and could be explained by the involutive changes in these muscles that are observed with aging as a consequence of different causes such as malnutrition or sarcopenia [2]. Taken together, we hypothesize this reduction in SSF with age in healthy volunteers parallels the decrease in the pharyngeal sensory function of these persons.

Regarding the SSF in patients with post-stroke OD, no significant differences were found when compared to the GIII HV group (HV of the same age). In contrast to what was previously described [165], [260], we did not observe significant differences in SSF between HV and post-stroke patients with OD due to the reduced number of patients included in the study and also to the combination of acute and chronic patients. In contrast, we observed significant changes in the EMG metric, with a significant increase in the amplitude, the duration and the AUC of the muscular contraction associated with each swallow. The increased duration of each swallow could be related to the prolonged neurophysiological and biomechanical response (time from GPJO-LVO) of swallowing reported in post-stroke patients [133]. In addition to a delayed oropharyngeal and prolonged swallow response, these patients showed a loss of symmetry of the pharyngeal sensory evoked potentials and their cortical representation[82] and reduced and delayed pharyngeal motor evoked potentials [136]. Regarding the increase in amplitude, a few studies have related the increase in this variable in older patients with OD to the loss of adipose tissue, specifically in the submental region, as fat attenuates the EMG signals [259], [261]. Another interpretation could be that these patients with post-stroke OD require a stronger muscular effort to elicit a spontaneous swallow.

The hypothesis that the neural control of spontaneous swallowing depends on the CPG, and could be similar to that described for the blink reflex [11], [80], [262], [263], leads to peripheral neurostimulation strategies as a possible treatment to improve SSF. One of the most used chemical strategies to improve swallow response is capsaicin stimulation. This natural agonist activates TRPV1 receptors, which are widely located in the epithelial cells and nerve endings of the human oropharynx and could be activated by endogenous or exogenous agonists [57], [59]. After acute treatment with capsaicin 10µM, post-stroke patients showed a significant increase in SSF without any major changes in the EMG metrics, probably due to the small sample. In a previous study in healthy volunteers, significant changes in pharyngeal and UES function were assessed by manometric and EMG metrics after acute treatment with capsaicinoids at the same concentration as used in our study [220]. Our group also has demonstrated the therapeutic effect of capsaicin at different concentrations and acute/subacute administration. Regarding the acute (single dose) treatment, we observed an improvement in time to LVC and UESO, reduction in the prevalence of oral and pharyngeal

residue and increased cortical excitability at a concentration of 150µM but not at 10µM [218], [245], [246]. The low dose showed better results when patients received the treatment during 10 days, 3 times a day. In this case, patients not only showed improvements in biomechanics but also in neurophysiology, inducing a faster and more intense response by shortening the latency and increasing the amplitude of the pharyngeal sensory evoked potential peaks. In addition, we described a significant correlation between the improvement of N1 peak latency and the improvement of time to LVC, suggesting that neuroplasticity processes were being induced that resulted in improved OSR [246], [252]. With this evidence we can conclude that capsaicin would induce greater conduction of stimuli through the afferent pathways to the CPG resulting in an enhancement of the SSF.

This proof-of-concept study had some limitations. First of all, the sample size of patients with post-stroke OD is quite small, combining patients in the acute and chronic state, and swallowing function was only assessed with V-VST. We are working on another prospective study where a larger sample of patients with an acute stroke and OD will be included as well as a group of acute post-stroke patients without OD. Further studies are needed to explore the SSF in OD patients to know whether it could be used as a screening tool. Finally, it would be interesting to study whether there are changes in the concentration of salivary SP after capsaicin treatment and if it is related to the improvement observed in SSF. Also, the duration of the improvement in SSF following TRPV1 stimulation with capsaicin should be measured.

Spontaneous swallowing frequency ranged around one swallow per minute in healthy young volunteers, which was significantly affected by age but not by gender. Post-stroke patients with OD showed no differences in SSF compared with HV of similar age but presented higher amplitude and longer duration of each spontaneous swallow. Acute stimulation with capsaicin resulted in a significant increase in SSF, suggesting the involvement of oropharyngeal TRPV1 receptors on the swallowing reflex control and further supports a potential pharmacological strategy to treat post-stroke patients with OD with these pharmacological compounds.

Chapter 2: A comparative study on the effect of acute pharyngeal stimulation with TRP agonists on the biomechanics and neurophysiology of swallow response in patients with oropharyngeal dysphagia

This study aimed to compare the therapeutic effect of six compounds of three families of TRP agonists (TRPV1, TRPA1, TRPM8) and two types of TP (Ms, XG) in order to discover the optimal components to develop an active pharmacological treatment for OD. The main results showed

that both TRP agonists and TP strongly reduced the prevalence of safety impairments through very different mechanisms of action. While the high viscosity levels achieved with TP protect the patient from penetrations and aspirations by reducing the mean bolus velocity and delaying LVC and UESO, TRP agonists protect patients by speeding bolus velocity and the neurophysiology of swallowing and reducing time to LVC and UESO at much lower viscosity levels. Our study shows TRPV1 and TRPV1/A1 agonists have the greatest potential to develop an active pharmacologic treatment to rehabilitate the swallowing function of patients with OD, and that an optimal strategy might be to combine them with the compensatory effects of fluid thickening at 250 mPas·s with XG TP.

The use of TPs to compensate for the biomechanical deficits that patients with OD have is and has been a largely-used and well-accepted therapeutic strategy by scientific societies [188]. Due to the high prevalence of impaired safety of swallow with thin liquid in several phenotypes of patients with OD (from 50.0% in Parkinsons' disease patients to 80.6% in patients with head and neck cancer) [173], [187], the use of TPs to avoid penetrations and aspirations into the airways has become standard therapy for patients with dysphagia. Other studies than the ones analyzed in this manuscript also indicate that TPs have a viscosity-dependent effect, meaning that as viscosity is increased, safety of swallow improves [173], [187], [188]. However, higher viscosities also increase the presence of oral or pharyngeal residue, especially in starch-based thickeners [188]. In addition, treatment compliance at high viscosity levels is very poor due to patients' dissatisfaction with taste and texture, particularly for MS [193]. Another key element to their effectiveness is shear rate and salivary alpha-amylase resistance, as both phenomena can cause viscosity to be significantly reduced in the oropharynx, increasing the risk of aspiration [191]. In this study, we have described the strong therapeutic effect of TPs and the differences in their mechanism of action when compared with TRP agonists. TPs do not improve biomechanics nor neurophysiological parameters such as time to LVC or UESO or latency and amplitude of pSEPs, and thus, they do not improve swallowing physiology, condemning the patient to permanent use of these products with their associated low adherence due to the low palatability, especially those patients with more severe OD who require higher viscosity levels. Moreover a previous study showed that higher viscosities could even increase the time to LVC and UESO and decrease bolus velocity [185]. Some studies that have tested new generation TPs composed with gums have found that the therapeutic range between the minimal and maximal protection is between 250 and 1000mPa·s [173], [187], a range that differs significantly from those previously used by starch-based products in which the highest viscosity was >3500mPa·s [218]. In addition, XG TP were

not affected by amylase in the oral phase. This improvement in TPs development might increase the compliance and reduce patient complications but would not change their compensatory mechanism of action.

On the other hand, our study also showed how the supplementation of a nectar viscosity bolus with the TRPA1/V1 agonist piperine in a concentration of 1mM had a similar therapeutic effect on safety of swallow as TP at high viscosity levels. We have found that in 50% of patients treated with piperine 1mM, the prevalence of safety impairments was reduced by at least 25%, whereas increasing bolus viscosity with TP reduced it by 20-27%. The effect of TRP stimulants was coupled with a significant improvement in the timing of the OSR, meaning this is an active therapy that improves swallowing physiology. As shown in our previous studies, the acute and sub-acute stimulation with TRP agonists did not only improve the PAS score, but also reduced the time to UESO and to LVC [218], [224], [226], [246], [247], this last being the main mechanism that protects the respiratory airway when swallowing [20]. Our comparative study showed that capsaicin (TRPV1) and piperine (TRPV1/A1), both at 150µM, produced the greatest reduction in the time to LVC and to UESO, and capsaicin 10µM, menthol 1mM and CIT-ISO were the TRP agonists with no acute effect on these parameters. As we stated in our previous studies, a lower concentration of capsaicin (10µM) and menthol (1mM) were not able to induce acute changes in either the biomechanics or in the neurophysiology of swallowing. Regarding capsaicin, we found in other studies that if the treatment was maintained for 10 days, it significantly reduced the time to LVC and the PAS score [246], and also increased the concentration of the neuropeptide substance P in saliva [222]. It has been also described that combininga TRPA1 and TRPM8 agonist, like CIT-ISO, can diminish the therapeutic effect they would have when applied separately [247], [251]. In a previous in vitro study using capsaicin (10^{-6}M) and piperine (10^{-3}M) in a bioassay using human PC-3 cells, we observed some desensitization effects after repetitive stimulation with these TRP stimulants [264]. However, in the present study in humans, we did not observe in vivo any desensitization process when comparing responses to TRP agonists at T1 and T2. Also in this comparative study, capsaicin 150µM was the TRP agonist that showed the strongest therapeutic effect on the biomechanics of the swallow response, as 50% of patients reduced the time to LVC by at least 100ms, showing a huge biomechanical improvement after TRPV1 stimulation on swallow physiology.

Finally, the effect on the neurophysiological response showed that only CIN-Zn (TRPA1 agonist) significantly reduced the latency of P2 peak, indicating that this could improve the integration of the sensory inputs in the brain that could be associated with the improvements

observed in the biomechanical response with the same agonist. This association between the improvements of both responses was observed in a previous study of our group, where we described a positive correlation between the improvements in the latency of N1 peak and the time to LVC after the stimulation with capsaicin 10µM for 10 days suggesting that TRP stimulation induced neuroplasticity processes which were translated into biomechanical improvements [246]. However, all TRP agonists seemed to have a positive effect on the neurophysiological response although they were not significant. When analysing the therapeutic effect through pharmacodynamics, as expected, only CIN-Zn showed a high therapeutic effect as 50% of patients shortened the latency of P2 peak by 21ms. In contrast, when performing the one-phase decay curve taking into account the changes induced in the N2-P2 amplitude, only capsaicin 10µM increased it, meaning that 50% of patients treated with this agonists in a low concentration improved it by 2µV.

We recognize our study has many limitations. The first one was the small sample size of some of the TRP agonists studies and the concentration tested. In addition, the only data on the effect on the neurophysiology is for capsaicin 10µM, CIN-Zn, CIT and CIT-ISO, the effect of the other TRP agonists on the neurophysiological response was not measured. In addition, although all studies have the same experimental design facilitating the interpretation of our results, only data from acute studies were included, since evidence of the sub-acute effect is not yet known for all agonists. Further clinical studies are needed in order to know exactly which TRP agonist has the best mid and long-term therapeutic effect on improving the biomechanics and neurophysiology of swallowing without inducing desensitization. Finally, some basic studies are also needed to evaluate the mechanism of action of each TRP agonist.

In conclusion, this comparative study showed that TRP agonists, in contrast to thickening products, have the potential to induce improvements in the neurophysiology, the biomechanics and the prevalence of unsafe swallows. TRPV1 and TRPV1/A1 agonists on the one hand and XG TP on the other seem the best candidates to develop new products for dysphagia patients by combining the strong therapeutic effects of both strategies. Understanding the specific mechanism of action of these compounds will help determine the optimal concentrations of agonists and the optimal viscosity values to reach this unmet need. The development of this new generation XG-based TP plus TRPV1 or TRPV1/A1 agonists will help to further move dysphagia treatment from compensation to the recovery of swallowing function.

Chapter 3: Acute and subacute effects of oropharyngeal sensory stimulation with TRPV1 agonists in older patients with oropharyngeal dysphagia: a biomechanical and neurophysiological randomized pilot study

Results from this study further confirm that the biomechanical airway protection mechanisms during the swallow response (mainly LVC) are delayed in older patients with OD and the cortical activation to pharyngeal sensory stimuli at pSEP are also delayed, impaired and reduced in this population. We also found that acute treatment with low doses of capsaicinoids (10µM) did not have any effect on the OSR or the pSEP. In contrast, sub-acute treatment with the same concentration of capsaicinoids induced significant cortical changes that correlated with significant improvements in the OSR of older patients with OD, further suggesting that the use of sensory stimulation by TRPV1 agonists could be a valid pharmacological strategy for these patients. However, although our results corroborate our initial hypothesis, we need to perform further studies and a randomized clinical trial with more patients to confirm that these neuroplastic and biomechanical changes in swallow function are caused specifically by sub-acute TRPV1 stimulation.

Our research strategy first characterized the impaired biomechanics of the OSR in older people using VFS and then the therapeutic effect of oropharyngeal sensory stimulation using TRP agonists. We found that impaired safety of deglutition and aspirations in older people are mainly caused by delayed LVC [119]. We also found that acute oropharyngeal sensory stimulation with natural capsaicinoids (150µM) had the strongest therapeutic effect over other TRP agonists by significantly reducing the prevalence of penetrations, pharyngeal residue, time to LVC and increasing bolus velocity in older OD patients [218], [226]. However, in the present study we did not find such an improvement in VFS results when applying acute stimulation with capsaicinoids at lower doses. This lower concentration (10µM) compared to the previous study (150µM) [218]was offered because of the poor palatability felt by patients at the higher concentration due to pungency, and the good results found in a previous sub-acute study with 10µM doses on older patients with OD with a positive responder rate of 68.42% [252]. Moreover, the application of the same reduced concentration of capsaicinoids for 10 days significantly reduced the severity of impaired safety alteration measured by the PAS by 24.15% (P=0.038) and the most relevant biomechanical element of the reconfiguration phase of the OSR, the LVC (P=0.042) (~100 ms reduction). In addition we found a responder rate of 71.43%, similar to that of our previous sub-acute study [252], further suggesting the strong therapeutic effect of this sensory-stimulation approach to develop future pharmacological treatments for OD.

We previously found that impaired conduction/integration of the afferent pathway in cerebral structures involved in sensory pharyngeal processing was closely associated with the pathophysiology of OD in older patients [86]. The preservation of the afferent pathway is essential for a safe and effective swallow, allowing continuous oropharyngeal feedback to higher-level cerebral centers and activation of sensorimotor integration processes [265]. In previous studies with a similar methodology, we found that older patients with OD had impaired cortical response to the pharyngeal electrical stimulus compared with older people without dysphagia. The amplitude of all peaks was clearly inferior in older patients with OD, while only the latency of N1 and N2 peaks was delayed [86], with values quite similar to those observed in the present study. We did not find any significant effect of acute stimulation on the latency or amplitude of pSEPs. In contrast, when we analyzed pSEP in the sub-acute study, we also found a significant reduction in the latency of the N1 peak, and an increase in the amplitude of the P1-N2 and N2-P2 and changes in cortical activation after capsaicinoids treatment, indicating an improvement in conduction (N1 and P1 peaks) and integration (N2 and P2 peaks) of sensory information into the cortex. This suggests that capsaicinoids treatment induces plastic cortical changes that are translated into a safer and faster OSR.

As we reported in a previous study, older patients with OD show an increase in prefrontal and associated-area activation to compensate for the impaired activation of the other brain areas involved in the OSR [86]. When we analyzed brain activity with sLORETA, we found a similar increase in frontal activation prior to the stimulation treatment. After acute stimulation, we found a reduction in brain activity in non-relevant areas for deglutition. However, after sub-acute capsaicinoids stimulation, we observed more activity in the cingulate gyrus (limbic lobe) at N1 and P2 peaks and of the medial frontal gyrus (frontal lobe) at N2 peak. Both areas, especially the cingulate gyrus, are mainly activated during swallowing preparation and play a major role in the perception of stimulus and go/no-go decisions [266], suggesting a neuroplasticity potential for future OD treatments based on sensory-stimulation.

We also found a strongly significant correlation between reduction in time to LVC and N1 pSEP peak latency reduction, indicating that biomechanical improvements seen with VFS correlate with neurophysiological improvements on the sensory side. On the other hand, we did not find this correlation when we compared the total duration of swallow with the same peak latency. This result agrees with a previous publication of our group which found that, after the application of acute capsaicinoids treatment, the time to LVC was significantly reduced but the treatment did not modify the total duration of OSR [218]. This indicates this

stimulant affects the first phase of pharyngeal swallow (airway protection mechanisms and reconfiguration from a digestive to a respiratory pathway).

Other studies have also found a close relationship between sensory deficits and impaired OSR, indicating that reduced sensory input is translated into an impaired motor response in patients with OD [26], [82]. This pathophysiologically-relevant factor in OD has been shown to be reversed or improved with our treatment, suggesting the relevance of sensory stimulation in the management and treatment of older patients with OD and indicating that sub-acute administration of capsaicinoids are a valid strategy to induce cortical changes that have a positive impact on swallow physiology. Despite these good results, we still need to adjust the dosage to obtain the highest biomechanical improvements with the minimal dose-effect due to the pungency of capsaicinoids. It is also important to note that there were no desensitization effects after two weeks of treatment.

Finally, there are some studies that correlate the increase in substance P (SP) level in saliva after pharyngeal sensory stimulation [233]. A recent publication showed that after oral capsaicin treatment (0.7µg during 7 days) there was an increase in salivary concentration of SP that was associated to an improvement in the safety and efficacy of swallowing, and to a shortening in the OSR in older OD patients [222]. These results suggest that SP can be used as a biomarker for neurostimulation treatment response to stimulants such as capsaicin and will be an interesting factor to take into account in future studies.

This study has some limitations, the main one being that it was a proof of concept with few patients in each group. Further studies with larger sample sizes are needed to reaffirm these results and with a second post-treatment neurophysiological evaluation at a longer follow-up time period to assess whether the observed neuroplasticity is maintained over time. In addition, patients from the acute study had lower severity of OD compared with those in the sub-acute study and this could have affected the results as the patients from the acute study had less potential improvement margin than those from the sub-actue. To solve this limitation, in future studies with a similar design, we will randomize patients to the acute or sub-acute group instead of randomizing only for treatment or placebo to balance the severity of OD between both therapeutic groups.

We have shown that impaired pharyngeal sensory function is a key element in the pathophysiology of dysphagia in older patients and treatments increasing the sensory input, such as TRPV1 agonists, will play a major role in future treatment of dysphagia. The acute

effect of capsaicin and capsaicinoids has been well studied by our group but the chronic effect is not well known and this study shows an improvement in this direction.

Future studies with larger patient samples, and including salivary SP measurement, are needed to better know the chronic effects of these stimulants and to select the most appropriate dose to improve dysphagia and avoid nutritional and respiratory complications among older patients with OD. The therapeutic paradigm for older patients with OD is now changing from compensatory to those that improve brain and swallow function.

Chapter 4: Oropharyngeal dysphagia in older people is associated with reduced pharyngeal sensitivity and low substance P and CGRP concentration in saliva

The aim of this study was to assess the relation between salivary SP and CGRP concentration and pharyngeal sensitivity in order to describe a peripheral biomarker in saliva to monitor pharyngeal sensory function in patients with OD and to identify potential "responders" to neurostimulation sensory treatments. The main results concur with our hypothesis as we have clearly observed that the salivary concentration of SP and CGRP were reduced and inversely correlate with pharyngeal sensory threshold, but only SP identified those patients with higher pharyngeal sensory threshold. We also observed that in addition to their pharyngeal sensory deficits, elderly patients with OD present a severe delay in the swallow motor responses involved in airway protection, with sarcopenia and hydropenia as main contributors to muscular dysfunction.

Participants in our study were classified in three groups (healthy volunteers, healthy elderly, elderly with OD) showing similar distribution between genders. Older patients in both groups show quite preserved functional and nutritional status and low number of comorbidities. However, our bioimpedance results in healthy elderly and elderly with OD showed increased fat mass and reduced lean, muscular mass, and cellular mass. Both older groups also showed a reduction in the intracellular water compared to healthy volunteers indicating that they had hypertonic dehydration. This hypertonic dehydration might be caused by decreased water intake due to multiple factors, such as reduced thirst sensation and side effects of some drugs, and also OD. This hyperosmotic stress causes cell dehydration which alters different physiological processes, and further contributes to frailty and functional decline in older people [137]. In addition, cell dehydration compromises the integrity of the plasmatic membrane, which alters its capacity to separate charges of the bioimpedance electric current and causes the reduction of the phase angle, as seen in our results. Phase angle is also related to the quality of the muscle function in older adults [267], [268]. Although

there is no gold standard for the study of hydration status [269], our study shows a significant reduction in intracellular water and muscle mass in elderly with OD confirming both "sarcopenic" and "hydropenic" pathophysiological elements in the weak muscular swallow response of our older participants. Also, healthy elderly and elderly with OD showed a reduction in the volume of saliva, with a strong significant positive correlation with phase angle. These results clearly show that the dehydration observed in our patients reduces saliva secretion and this might also contribute to OD [270]. These results match with a previous study of our group where patients with chronic OD showed high prevalence of sarcopenic malnutrition and intracellular dehydration [146] clearly showing the effect of OD on body homeostasis in older people and the involvement of these two elements in the pathophysiology of "sarcopenic" and "hydropenic" dysphagia.

We assessed swallowing function through VFS, showing that the older patients included in the elderly with OD group had high prevalence of efficacy and safety impairments, a mean maximum PAS of 4.38±0.77 and delayed time to LVC and UESO. As shown previously, a delayed swallow response, specifically the time to LVC, is related to impaired safety of swallow and penetrations and aspirations into the airway [119]. In addition, our patients showed a significant decrease in muscle mass. It is already known that there are age-related changes in the swallowing muscles, including tongue and cervical muscles, which are also related to OD and defined as "sarcopenic dysphagia" [147], [271]–[273]. This phenomenon could explain the high prevalence of oral and pharyngeal residue in both older groups in our study. It is important to note that dehydration and sarcopenia are not only a cause but also a consequence of OD due to the secondary malnutrition and low fluid intake [272].

Previous studies from our group showed that older patients with OD presented an impairment in the oropharyngeal sensory pathway and impairments in the cortical sensory activation that was reflected in the lengthening of the latency and the reduction of the amplitude of the characteristic peaks of the pSEPs [86]. The aging process leads to a reduction of pharyngeal sensitivity assessed with the pharyngeal sensory threshold to electrical stimulation, which is significant in older patients with OD. Up to 35.71% of patients classified in the elderly with OD presented a pharyngeal sensory threshold above the reference interval and showing an impaired pharyngeal sensitivity. The loss of sensory function correlates with peripheral neurodegeneration and a reduction of the oropharyngeal mechanoreceptors in an aging animal model, altering chewing and swallowing functions as a consequence [256]. In human adults, a reduction of the number of small myelinated fibers of the SLN has also been described [250]. In addition, our group also described a correlation between the latency of the

N1 peak of the pSEPs and the time to LVC in older patients with OD [246], suggesting that there is a relationship between the impairment of the pharyngeal sensory function and the slow biomechanics of the airway protection mechanisms (time to LVC). Our present study adds a potential biomarker for this sensory dysfunction as salivary SP has been found to be reduced in this population.

We have also previously described the localization of the TRP and ASIC receptors in the human oropharynx: TRPM8 and ASIC3 are found on submucosal sensory nerves in the human oropharynx; and that TRPV1 and TRPA1 are widely expressed with two distinct patterns: TRPV1 was localized at epithelial cells and nociceptive fibers, in contrast TRPA1 was localized below the basal lamina [57], [58]. We believe that the activation of all these receptors with chemical and thermal bolus stimuli might cause the secretion of SP and CGRP to saliva. Some studies have already shown an increase in the concentration of SP and CGRP in saliva after intrapharyngeal sensory electrical stimulation or capsaicin [221], [222], [233]. In addition, our group showed that patients with OD had lower basal concentration of SP than healthy participants of the same age and sex [274], and studies showed that patients with OD with less salivary SP had a lower spontaneous swallowing frequency [65]. In the present study we observed that concentrations of both SP and CGRP in saliva moderately but significantly correlated with the pharyngeal sensory threshold. This correlation has allowed us to divide the population according to the salivary SP and CGRP concentrations they present, showing that those patients with low concentrations of SP have a higher sensory threshold and vice versa. This was not the case for CGRP, which was severely reduced in all older patients and did not allow us to discriminate the impairment of pharyngeal sensitivity based on its concentration in saliva. Therefore we propose salivary SP as a peripheral biomarker for oropharyngeal sensory impairment. We have demonstrated the therapeutic effect on swallowing function of different peripheral and central sensory stimulation strategies in several studies [218], [224], [246], [247], [252]. The dynamics of the concentration of SP in saliva before and after the stimuli would indicate which patients respond or not to peripheral sensory neurostimulation treatments.

This study has some limitations. Our sample is small and only includes independently-living older people with mild OD. Further studies are needed to know if this alteration in salivary SP and CGRP is observed in older patients with more severe forms of OD and in patients with OD due to other causes such as stroke or neurodegenerative disease. In addition, studies following sensory stimulation should also demonstrate whether there is an increase in the level of these peptides in the patients that “respond” to these strategies.

In conclusion, this study shows that older people, especially the elderly with OD, have "sarcopenic", "hydropenic" dysphagia, reduced saliva secretion, reduced salivary SP and CGRP concentration and impaired pharyngeal sensory function with increased pharyngeal sensory threshold. We also demonstrate that there is a correlation between salivary SP and CGRP and pharyngeal sensory threshold in elderly patients with OD, but only SP could discriminate pharyngeal sensory threshold impairment according to its concentration in saliva, suggesting its potential role as a peripheral biomarker of impaired pharyngeal sensitivity in this dysphagic population.

GENERAL DISCUSSION

The present doctoral thesis includes several studies published or in the process of publication that had the aim to better understand the pathophysiology of OD and how stimulation with TRP agonists could be one of the best therapeutic strategies in rehabilitating the swallowing function. Independently of the timing of publication of each study, the main aim of the thesis is to develop a full strategy for a pharmacologic treatment of OD based on: a) identification of the candidates for pharmacological stimulation (patients with OD and impaired sensory function) by using the assessment of two neuropeptides in saliva (SP and CGRP) as peripheral biomarkers; b) assessment of the effect of pharyngeal sensory stimulation on the sensory cortex and the biomechanics of voluntary swallow, as well as the effect on stimulation of CPG in the brain stem, and the comparison of the pharmacodynamics of the therapeutic effect of acute pharyngeal stimulation with several types of TRV1, TRPA1 i TRPM8 agonists on the biomechanics and neurophysiology of swallow to select the TRP candidates for pharmacological treatment of OD; c) assessment of the neurophysiological and biomechanical effects of subacute stimulation with TRPV1 over two weeks; and d) the description of the physiological bases for optimal combination of the compensatory effects of TP based in XG with that of the most powerful TRV1 and TRPA1 agonists, to further develop new products for patients with dysphagia.

Chapter 4 of this doctoral thesis explores the relation between SP and CGRP and pharyngeal sensitivity in older patients with OD and healthy young and older people. We found that there was a reduction in the concentration of both neuropeptides in the saliva of older people with and without OD, only CGRP being significant when compared to healthy volunteers. Moreover, SP and CGRP had a negative correlation with pharyngeal sensory threshold to electrical stimulation, showing that the lower the salivary concentration of these neuropeptides, the higher the pharyngeal sensory threshold. However, only SP could probably

be used as a peripheral biomarker of the status of pharyngeal sensitivity as only it identifies participants with a significant impairment in the pharyngeal sensory threshold. These results could explain the impairments observed in the afferent or sensory pathway described in several publications [25], [82], [86] and also in this thesis. Our hypothesis is that the low salivary concentration of SP and CGRP observed in patients with OD is a biomarker and also contributes to the altered transmission of sensory inputs from the periphery to the pharyngeal sensory cortex and brainstem, impairing, as a consequence, the voluntary and spontaneous swallow response. As mentioned before, this mechanism could be similar to the one described for cough reflex, in which the secretion of SP (induced by endogenous or exogenous sensory stimuli) facilitates the transmission of the sensory inputs to the brainstem [68]. The main exogenous stimuli that induced the secretion of SP and CGRP were the TRP agonists. The colocalization of both neuropeptides and TRP receptors in several human tissues has been described [72], [74], [75], [77], [275]–[280] and their secretion into saliva is increased after oral stimulation with TRP agonists [220]–[222]. This information suggests the use of SP and CGRP also as peripheral biomarkers of TRP stimulation treatment responsiveness, opening up the possibility of personalized medicine.

In this doctoral thesis, the acute therapeutic effect of several TRP agonists has been evaluated in the search for a pharmacological treatment for OD. Our group has extensive experience in this field, as we first studied the localization and expression of TRPV1, TRPA1 and TRPM8 receptors in the human oropharyngeal mucosa [57], [58] and explored the concentrations needed of some natural TRP agonists, such as capsaicin and piperine, using an immunoassay performed with PC-3 cells [264]. With these first concentrations we have already performed several clinical studies evaluating the therapeutic effect on swallowing of several natural or pharmacological TRPV1, TRPV1/A1 and TRPM8 agonists [218], [224], [226], [245]–[247], [252], [281].

The aim of the first chapter was to evaluate the acute therapeutic effect of several TRPA1 and TRPA1-TRPM8 agonists on the biomechanics and neurophysiology of voluntary swallowing in patients with OD in the search for pharmacological stimulants of the swallow response. We observed that acute stimulation of TRPA1 receptors with CIN-Zn or CIT reduced the time to LVC and UESO, reaching similar results of what was already described in other studies where the effect of capsaicin (TRPV1 agonist), piperine (TRPA1/V1 agonist) and menthol (TRPM8 agonist) were evaluated [218], [224], [226]. It is important to note that the combined stimulation of TRPA1 and TRPM8 agonists using citral-isopulegol only reduced the time to UESO, probably due to a cross-desensitization between the two agonists [251].

Regarding the neurophysiological effect, only CIN-Zn reduced the latency of P2 peak, inducing a faster integration of the sensory input in the brain. In addition, the peripheral stimulation with this agonist increased brain activation, to a similar extent to that induced by capsaicin or piperine [223], [246]. In the first chapter, the acute therapeutic effect of TRPV1 agonists on the spontaneous swallow response was also described. Acute stimulation with capsaicin 10µM in post-stroke patients significantly increased the SSF reaching similar frequency to young healthy people, without major changes in electromyographic signals. As it is hypothesised that neural control of spontaneous swallowing depends on CPG, similar to the control of the blink reflex [11], [80], [262], [263], all the results explained in Chapter 1 suggest that the peripheral stimulation of TRP receptors with several natural agonists induces neurophysiological improvements by stimulating both the pharyngeal sensory cortex and the CPG, the two main pathways involved in the control of voluntary and reflexive swallow function (Figure 1).

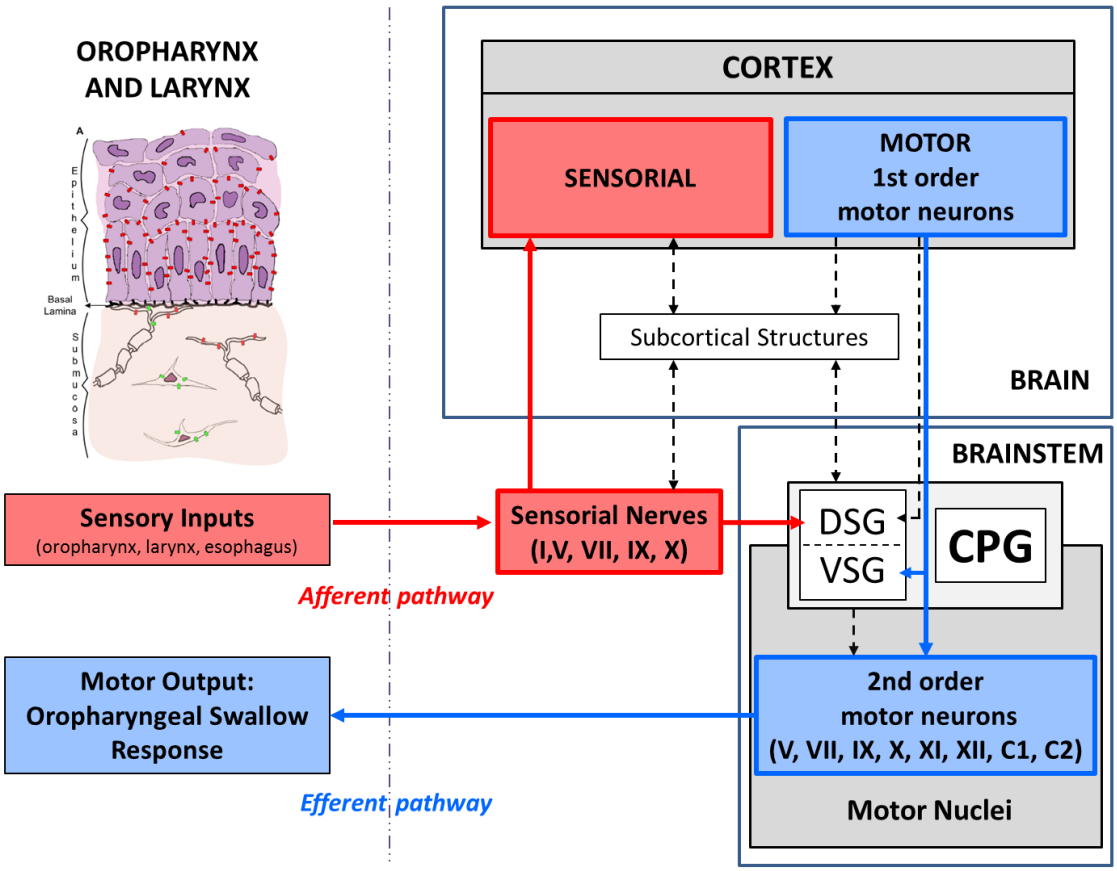

Figure 1: Pathways controlling voluntary swallow and spontaneous swallow as potential targets for TRP stimulation. Reproduced from Cabib et al 2016 [282].

The study included in Chapter 3 aimed to compare the acute and the sub-acute therapeutic effect of capsaicin 10µM. In a previous study of our group, where a concentration of 150µM of capsaicin was used, patients experienced pungency and some of them discomfort

during and after the treatment, despite the positive therapeutic effect observed on swallowing biomechanics [218]. Although not assessed formally, it is relevant to explain that in our initial studies, most patients preferred the pungency of 150µM of capsaicin to the further increase in viscosity by MS. Because of this pungency, we decided to reduce the concentration of capsaicin to a tenth, observing that acute stimulation with capsaicin 10µM was not sufficient to induce improvements in either the biomechanical or the neurophysiological response. However, if patients took the treatment three times a day for 10 days, we observed a significant improvement in safety of swallow with a reduction in PAS score and time to LVC, with a patient responders rate up to 70%, reaching similar therapeutic effect to what was already observed in other acute [218], [220] and sub-acute studies [222], [252]. Regarding the neurophysiological response, we observed a reduction in N1 latency and an increase in P1-N2 and N2-P2 amplitudes after the treatment, showing increased brain activation. In addition, this was the first study to observe a significant correlation between the improvements induced in the neurophysiological response (N1 peak latency) and the biomechanical one (time to LVC) after an active treatment. These results indicate that peripheral neurostimulation induce neuroplasticity that will be translated into improvements in the conduction of the stimuli from the pharynx to the cortex, and also in brain excitability, inducing a faster and more intense efferent response, as observed in other studies performed with TRPV1 agonists in post-stroke patients with OD [136]. Moreover, no desensitization process was observed, indicating that we are closer than ever to developing a safe and effective active pharmacological treatment for OD.

Finally, our aim in the second chapter was to assess which of the several TRP agonists explored by our lab had the highest therapeutic potential, and to compare the two main strategies for dysphagia treatment: thickened fluids (compensation) and pharmacological stimulation. As the the main clinically relevant neurophysiological parameter for the swallow response has still not been describedç, we selected the improvements induced on time to LVC (which is known to be the main mechanism that protects the airway during swallowing) to identify which TRP agonist had the greatest therapeutic potential:

TRPV1 agonists ≈ TRPV1/A1 agonists > TRPA1 agonists > TRPM8 agonists

In addition, we observed that thickening products, independently of their composition, improved the prevalence of safe swallows when the viscosity of the bolus was increased from nectar to pudding. However, acute treatment with nectar boluses supplemented with TRP agonists improved the prevalence of safe swallows and the biomechanical and

neurophysiological response. This comparative study also showed that the mechanisms of action of the two types of treatment worked in an opposite way: compensatory treatments at pudding viscosity reduce the prevalence of unsafe swallows by reducing the mean bolus velocity and increasing the time to LVC and UESO. It is important to highlight that at nectar viscosity the timing of OSR was not changed when compared to liquid, showing that the therapeutic effect is mediated by a purely compensatory mechanism at this medium viscosity level [185], [186]. As opposed to this finding, TRP agonists added to a nectar bolus also reduced the prevalence of unsafe swallows but through the reduction of time to LVC and UESO and increasing bolus velocity, in addition to shortening the latency of pSEP peaks and increasing brain activation.

FUTURE PERSPECTIVES

This doctoral thesis increased the evidence on the need to develop an active pharmacological treatment that allows the rehabilitation of the swallowing function in patients with OD. Our experience with both compensatory and active treatments leads us to suggest the development of a new treatment that combines these two strong therapeutic strategies, by adding TRPV1 or TRPV1/A1 agonists to 250 mPas·s boluses composed of xanthan-gum-based thickening products (Figure 2). This is another step leading dysphagia treatment from compensation to the recovery of swallow function.

It is important to note that TRP agonists are peripheral neurostimulation treatments that could induce desensitization processes when maintained over time. In order to avoid this effect it is important to perform more studies where the mid and long term effects of peripheral stimulation with TRP agonists will be assessed, in addition to studying which is the best therapeutic paradigm and dose. Moreover, although the therapeutic effect is known, the mechanisms of action at a molecular level still need to be discovered. Finally, more studies on the role of SP and CGRP neuropeptides are needed in order to describe a good peripheral and non-invasive biomarker to identify which patients could respond to these treatments without using invasive techniques.

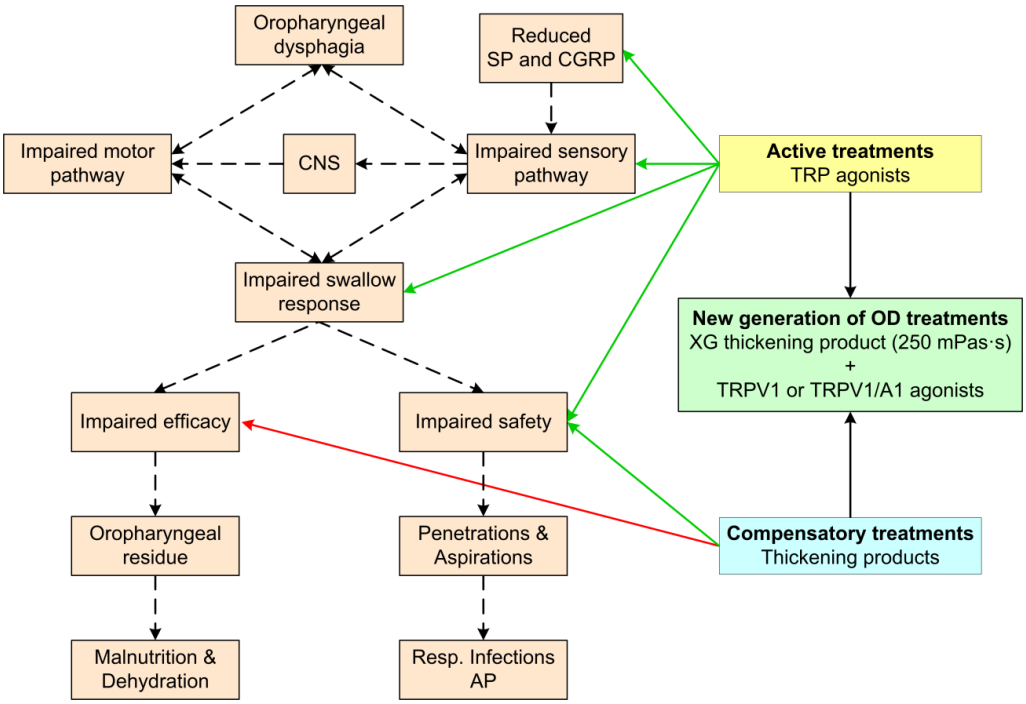

Figure 2: Schematic representation of consequences and complications of OD and present and future treatment perspectives. Red arrow: impaired; green arrow: improvements; SP: substance P; CGRP: calcitonin gene-related peptide; CNS: central nervous system; TRP: transient receptor potential; OD: oropharyngeal dysphagia; XG: xanthan gum

CONCLUSIONS

C1. TRPA1 stimulation with CIN-Zn or CIT improved the biomechanics of the swallow response which, in the case of CIN-Zn, was associated with a significant improvement in cortical activation and safety of swallow. Acute stimulation with the TRPV1 agonist capsaicin caused a significant increase in SSF, suggesting its potential role as a therapeutic strategy for CPG activation in patients with OD. These results provide the basis for the development of new active treatments for oropharyngeal dysphagia using TRP agonists.

C2. TRPV1 and TRPV1/TRPA1 agonists on the one hand and xanthan gum thickening products on the other are the best ingredients to develop new products for dysphagia patients, by combining the strong therapeutic effects of each. A new generation of xanthan-gum-based thickening products at 250mPas·s containing TRPV1 or TRPV1/TRPA1 agonists will help move dysphagia treatment from compensation to rehabilitation of the swallowing function.

C3. After 2 weeks of treatment, oropharyngeal sensory stimulation with capsaicinoids (TRPV1 agonist) improved the biomechanics, by reducing the prevalence of unsafe swallows and the time to laryngeal vestibule closure, and the neurophysiology of swallowing response, by shortening the latency and increasing the amplitude of pharyngeal sensory evoked potential peaks. Sub-acute stimulation also induced a neuroplasticity process that correlated with improvements in swallowing biomechanics, apparently without desensitization.

C4. Older patients with OD presented hydropenia and sarcopenia, reduced salivary substance P and calcitonin gene-related peptide and impaired pharyngeal sensitivity. Our results show substance P levels in saliva are a potential biomarker to predict pharyngeal sensitivity impairments in patients with OD.

Recently, the Nobel Prize in Physiology or Medicine was awarded to Dr. David Julius and Dr. Ardem Patapoutian for their discovery of the TRPV1 receptor. This event enhances the use of this receptor as a therapeutic target, among other TRP receptors. The results of the present doctoral thesis will contribute to the development of a new generation of treatments based on peripheral neurostimulation to treat several disorders related to sensory dysfunction.

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ANNEX

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ORIGINAL ARTICLE

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A randomized clinical trial on the acute therapeutic effect of TRPA1 and TRPM8 agonists in patients with oropharyngeal dysphagia

Noemí Tomsen[1,2,3] | Daniel Alvarez-Berdugo[1,2] | Laia Rofes[1,2] | Omar Ortega[1,2] | Viridiana Arreola[2] | Weslania Nascimento[2] | Alberto Martin[2] | Christopher Cabib[2] | Mireia Bolivar-Prados[2] | Lluís Mundet[2] | Coline Legrand[4] | Pere Clavé[1,2] | Stephanie Michlig[4]

[1]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Instituto de Salud Carlos III, Barcelona, Spain

[2]Gastrointestinal Motility Laboratory, Hospital de Mataró, Consorci Sanitari del Maresme, Mataró, Spain

[3]Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

[4]Nestlé Research, Lausanne, Switzerland

Correspondence
Pere Clavé, Department of Surgery, Hospital de Mataró, Universitat Autònoma de Barcelona, Carretera de Cirera s/n 08304, Mataró, Spain.
Email: pere.clave@ciberehd.org

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Abstract

Background: Oropharyngeal dysphagia (OD) treatment is moving away from compensatory strategies toward active treatments that improve swallowing function. The aim of this study was to assess the acute therapeutic effect of TRPA1/M8 agonists in improving swallowing function in OD patients.

Methods: Fifty-eight patients with OD caused by aging, stroke, or neurodegenerative disease were included in a three-arm, quadruple-blind, randomized clinical trial (NCT02193438). Swallowing safety and efficacy and the kinematics of the swallow response were assessed by videofluoroscopy (VFS) during the swallow of 182 ± 2 mPa·s viscosity (nectar) boluses of a xanthan gum thickener supplemented with (a) 756.6 μmol/L cinnamaldehyde and 70 μmol/L zinc (CIN-Zn) (TRPA1 agonists), (b) 1.6 mmol/L citral (CIT) (TRPA1 agonist), or (c) 1.6 mmol/L citral and 1.3 mmol/L isopulegol (CIT-ISO) (TRPA1 and TRPM8 agonists). The effects on pharyngeal event-related potentials (ERP) were assessed by electroencephalography.

Key Results: TRPA1 stimulation with either CIN-Zn or CIT reduced time to laryngeal vestibule closure (CIN-Zn P = .002, CIT P = .023) and upper esophageal sphincter opening (CIN-Zn P = .007, CIT P = .035). In addition, CIN-Zn reduced the penetration-aspiration scale score (P = .009), increased the prevalence of safe swallows (P = .041), and reduced the latency of the P2 peak of the ERP. CIT-ISO had no positive effect on biomechanics or neurophysiology. No significant adverse events were observed.

Conclusions and Inferences: TRPA1 stimulation with CIN-Zn or CIT improves the swallow response which, in the case of CIN-Zn, is associated with a significant improvement in cortical activation and safety of swallow. These results provide the basis for the development of new active treatments for OD using TRPA1 agonists.

KEYWORDS

deglutition disorders, dysphagia, sensory function, therapeutics, TRP agonists

Noemí Tomsen and Daniel Alvarez-Berdugo contributed equally to the study.

1 | INTRODUCTION

Oropharyngeal dysphagia (OD) is a recently recognized geriatric syndrome, with 11.4%-33.7% prevalence among independently living older people and up to 51% prevalence among institutionalized ones.[1-3] It is prevalent among stroke patients, affecting 40%-81%,[4-6] and patients with neurodegenerative diseases, with 35%-82% prevalence in Parkinson's disease and 19%-84% in Alzheimer's.[7,8] OD is a condition recognized by the WHO in the ICD-9 and ICD-10[9] under the category of "Symptoms and Signs involving the Digestive System" and causes severe complications such as malnutrition, dehydration, and respiratory infections, which significantly increase the mortality rate in these patients.[10,11] However, there is still no pharmacological treatment for OD. Current OD management is based on compensatory strategies including modifying bolus viscosity with thickeners, and postures and maneuvers, but compliance is poor and patients may lack the functional capacity to collaborate.[1]

OD can cause impairments in both efficacy and safety of swallow. Signs of impaired efficacy, such as oral and pharyngeal residue, are caused by inadequate bolus formation and weak tongue propulsion due to sarcopenia, weakened muscles, or impairments in the areas of the central nervous system that produce motor commands or in the neural efferent pathways conducting them.[11] Signs of impaired safety, such as penetrations and tracheobronchial aspirations, are mainly caused by delayed swallow response.[12] The oropharyngeal swallow response (OSR) is triggered by oropharyngeal sensory inputs that reach the central pattern generator of the brainstem and are modulated by signals from the sensory and motor cortex and subcortical nuclei.[13,14] Thus, oropharyngeal sensory perception plays a major role in the swallow response.

Previous studies have shown that older patients and those with stroke and neurodegenerative diseases had impaired oropharyngeal sensory function which correlated with swallow safety impairments and aspiration.[15-17] Our group has published several studies that showed that pharyngeal sensory evoked potentials (ERP) in older and stroke patients with OD present lower amplitude and longer latency than in healthy volunteers or older and stroke patients without OD. These impairments imply that patients with OD present impaired cortical activation patterns or disrupted afferent communication between the pharynx and the sensory cortex.[18,19] The reduction in oropharyngeal sensitivity can be targeted to treat patients with OD.

Several groups have used sensory stimulants to treat OD, aiming to increase oropharyngeal sensory stimulation to reach a patient's sensory threshold and trigger a proper swallow response.[20,21] This can be achieved with thermal,[22-24] mechanical,[25] and chemical[26-29] stimuli usually through activating transient receptor potential channels (TRP) particularly TRPV1, activated by capsaicin,[30] TRPA1, by noxious cold and chemical substances such as allyl isothiocyanate (garlic),[31] and TRPM8, activated by mildly cold and chemical substances such as menthol (peppermint).[32]

In previous studies, we located TRP channels in human oropharyngeal mucosa, finding that TRPV1 was expressed within epithelial cells of the oropharynx; TRPA1, in sparse cells and submucosal structures below the basal lamina, while TRPM8 was found on submucosa sensory nerves. The activation of these TRP receptors increase sensory input through the release of neuropeptides such as calcitonin gene-related peptide (CGRP) or substance P (SP).[33,34] In clinical trials,[34-36] we found that activation of TRPV1 with capsaicinoids reduced the prevalence of both safety and efficacy impairments by shortening the time to laryngeal vestibule closure (LVC) and increasing bolus velocity.[34] We also proposed the combination of a new generation of xanthan gum-based thickeners with peripheral neurostimulants (TRP agonists) to reduce the therapeutic viscosity needed by patients and thus improve compliance.

The main objective of this study was to test the therapeutic effect of three TRPA1 and/or TRPM8 agonists in combination with a xanthan gum-based thickener on VFS signs of safety of swallowing and the OSR and cortical activation (ERP) in older patients and in patients with stroke and neurodegenerative disease with OD. The agonists tested were citral and cinnamaldehyde with zinc for TRPA1 and isopulegol for TRPM8.

Key Points

- Patients with oropharyngeal dysphagia (OD) caused by aging or neurological disease present a decline in pharyngeal sensory function.
- TRP receptors are widely expressed in the epithelial cells and sensory nerves in the human oropharynx.
- We assessed the acute effect of three TRP agonists and found an improvement in biomechanics and neurophysiology, translated into a faster oropharyngeal swallow response, a shortening of the latency of the pharyngeal sensory evoked potentials, and an increase in cortical activation, particularly with TRPA1.
- Our results provide the basis for the development of new active treatments for OD using TRPA1 agonists.

2 | METHODS

2.1 | Patients

This study was a quadruple-blind (participant, care provider, investigator, outcomes assessor) randomized interventional crossover trial with a hybrid design in which participants were randomized to one of the three, parallel groups of each active ingredient. The treatments were compared with a control treatment with no agonist, and all were given in 5 mL boluses at 182 ± 2 mPa·s viscosity at 50 s^{-1}, at 25°C (nectar according to the NDD[37]) achieved with 1.2 g/100 mL of the xanthan gum-based thickener Resource® Thicken Up Clear (Nestlé Health Science). The study was approved by the Ethical Committee of the Hospital de Mataró and performed according to the rules and recommendations of the Declaration of Helsinki and

subsequent amendments. This clinical study was registered with the code NCT02422576. More details on sample size calculation and randomization can be found in the Appendix S1.

The study was performed on patients referred to our outpatient dysphagia clinic at Hospital de Mataró who signed the informed consent. Patients were recruited between March 2015 and May 2017 and screened with the volume-viscosity swallowing test (V-VST), a validated bedside clinical test,[38] and those who fulfilled the inclusion criteria were randomized into treatment groups. The inclusion criteria were accepting to participate and signing informed consent, age >55 years, signs of OD during the V-VST (cough, oxygen desaturation ≥3%, or voice change), and that the cause of OD was neurodegenerative disease, stroke (>3 months), or aging. The exclusion criteria were history of head and neck radiotherapy for cancer, allergy to investigational products (IPs) or iodine radiopaque solution, severe respiratory disease requiring oxygen supplementation, recent history of surgical intervention, current alcohol abuse (more than 2 drinks/d), incapacity to complete the study, involvement in another clinical trial currently or in the last month, damage or irritation of the oropharyngeal mucosa, having a pacemaker or electrode implants, or epilepsy. Patients were also excluded if they had no signs of impaired safety of swallow (Penetration-Aspiration Scale (PAS) ≤ 2) or a PAS = 8 during the first nectar viscosity series (T0) of the videofluoroscopy (VFS).

2.2 | Investigational products (IP)

The IPs tested in this clinical trial were (a) cinnamaldehyde (756.6 μmol/L)/(100 ppm) and zinc (70 mmol/L) as mild-flavored TRPA1 agonists,[39] (b) citral (1.6 mmol/L)/(250 ppm) as a lemon-flavored TRPA1 agonist, and (c) citral (1.6 mmol/L)/(250 ppm) and isopulegol (1.3 mmol/L)/(200 ppm) as a mix of lemon-flavored TRPA1 agonist and cooling TRPM8 agonist.

2.3 | Experimental design

The study design included 4 visits. On Visit 1, patients were screened with the V-VST for clinical signs of OD and clinical and demographic data were collected. On Visit 2, 7 days later, the patient was randomized into a treatment group and given a VFS which consisted of a control series (T0) with 5, 10, and 20 mL boluses of nectar viscosity (181.92 ± 1.75 mPa·s at 50 s^{-1}); after 2 minutes, another series of 5, 10, and 20 mL nectar viscosity boluses with the IP (T1); and after 5 minutes, another series of nectar viscosity boluses containing the IP (T2) (Figure 1). All the boluses contained iodine radiopaque solution Omnipaque™ (GE Healthcare) diluted in water in a proportion 1:1.

On Visit 3, 3-7 days after the VFS, the first EEG (EEG1) was performed and then, between 3 and 7 days after the EEG1, on visit 4, the second EEG (EEG2) was performed. During the EEGs, the ERP were evaluated before (control) and after two administrations of 35 mL nectar viscosity boluses containing the IP or 1/2000 of ethanol as placebo (T1 and T2) the order of which was also randomized. At the end of each EEG visit, the patient was asked to evaluate the intensity and pleasantness of the administered treatment (IP or placebo) on a 0-10 scale to assess the palatability of each compound, and any sign of gut discomfort was registered with a gut comfort evaluation test. The primary outcome measure of this study was the improvement in the safety of the swallowing function, according to the maximum PAS score obtained during the deglutition of 5, 10, and 20 mL nectar boluses (with and without active ingredient) during the VFS.

2.4 | Videofluoroscopy procedures

VFS was performed on all patients while seated in a lateral projection that included the oral cavity, the pharynx, the larynx, and the cervical esophagus. The VFS was obtained with a Super XT-20 Toshiba Intensifier (Toshiba Medical Systems Europe) and recorded at 25 frames/s for later analysis with a Canon 3ccd Digital Video Camcorder Xm2 (Canon Inc). The VFS signs of safety and efficacy impairments of swallow and the swallow biomechanics were analyzed with the software Swallowing Observer (Image and Physiology SL).[40] The VFS signs of safety impairment analyzed were the occurrence and severity of penetrations and aspirations prior, during and after swallow expressed as the % of patients with safe swallow (PAS = 1 during the three boluses of each series), as well as the PAS score according to the Rosenbek Scale.[41] The VFS signs of efficacy impairment analyzed were the observation of residues after swallow in the oral cavity, the pyriform sinus, or the vallecula. The OSR parameters analyzed were the timing to LVC, laryngeal vestibule opening (LVO), and upper esophageal sphincter opening (UESO) (Figure 2).[40] Mean bolus velocity (cm/s) and bolus propulsion forces (mN) were also assessed as previously described.[35,40]

2.5 | Electroencephalography procedure: pharyngeal event-related potentials (ERP)

Patients were studied by EEG during the application of several trains of electrical stimuli to their pharynx as described in a previous study.[18] The sensory and tolerance thresholds were determined by triplicate for each patient, and three sets of 100 squared wave 0.2 ms with an interstimuli interval of 5 seconds at 75% intensity of the tolerance threshold were used to assess the ERP before and after two administrations of 35 mL nectar viscosity fluid supplemented with the IP or the placebo (T1 and T2).

Cortical responses to electrical stimuli were recorded with a 32 scalp tin electrodes cap (Electro-Cap International Inc) connected to a BrainAmp amplifier (Brain Products GmbH) as described previously.[18,19] Once registered, the ERP were analyzed offline and processed with BrainVision Analyzer Software 2.0 (Brain Products), filtered, and corrected for eye blink. Based on the results of the ERP analysis, the time frames used to compute the cortical activity

distribution were 56-80 ms for the N1 peak, 120-150 ms for the P1 peak, 220-270 ms for the N2 peak, and 300-350 ms for the P2 peak. Finally, the ERP of each group of patients were averaged to obtain the grand average.

2.6 | Adverse events

During the study, all adverse events or serious adverse event were recorded and notified to the review board of the Hospital de Mataró.

2.7 | Data analysis

To analyze the demographics and the baseline swallowing physiology and clinical signs, all groups were compared with a Kruskal-Wallis test with Dunn's post-test for continuous variables or with a chi-squared test for categorical variables. To analyze the effect of each treatment, the results were compared with the placebo results; the McNemar case-control test was used to compare categorical variables and the Wilcoxon *t* test for paired non-parametrical variables was used for continuous variables. All continuous data were represented as mean ± standard deviation. To assess the differences in cortical localization of the ERP between pretreatment and post-treatment status for each IP and placebo, the sLORETA software package was used. The differences were computed voxel by voxel with a non-parametric *t* test between the control and each administration series (T1 and T2). The results correspond to maps of *t*-statistics for each voxel. A *P*-value < .05 was considered statistically significant.

The brain source of each ERP component was localized, as previously described,[18,19] using the standardized brain electromagnetic tomography (sLORETA) software.[42] Computations were made on a head model,[43] using the Montreal Neurological Institute

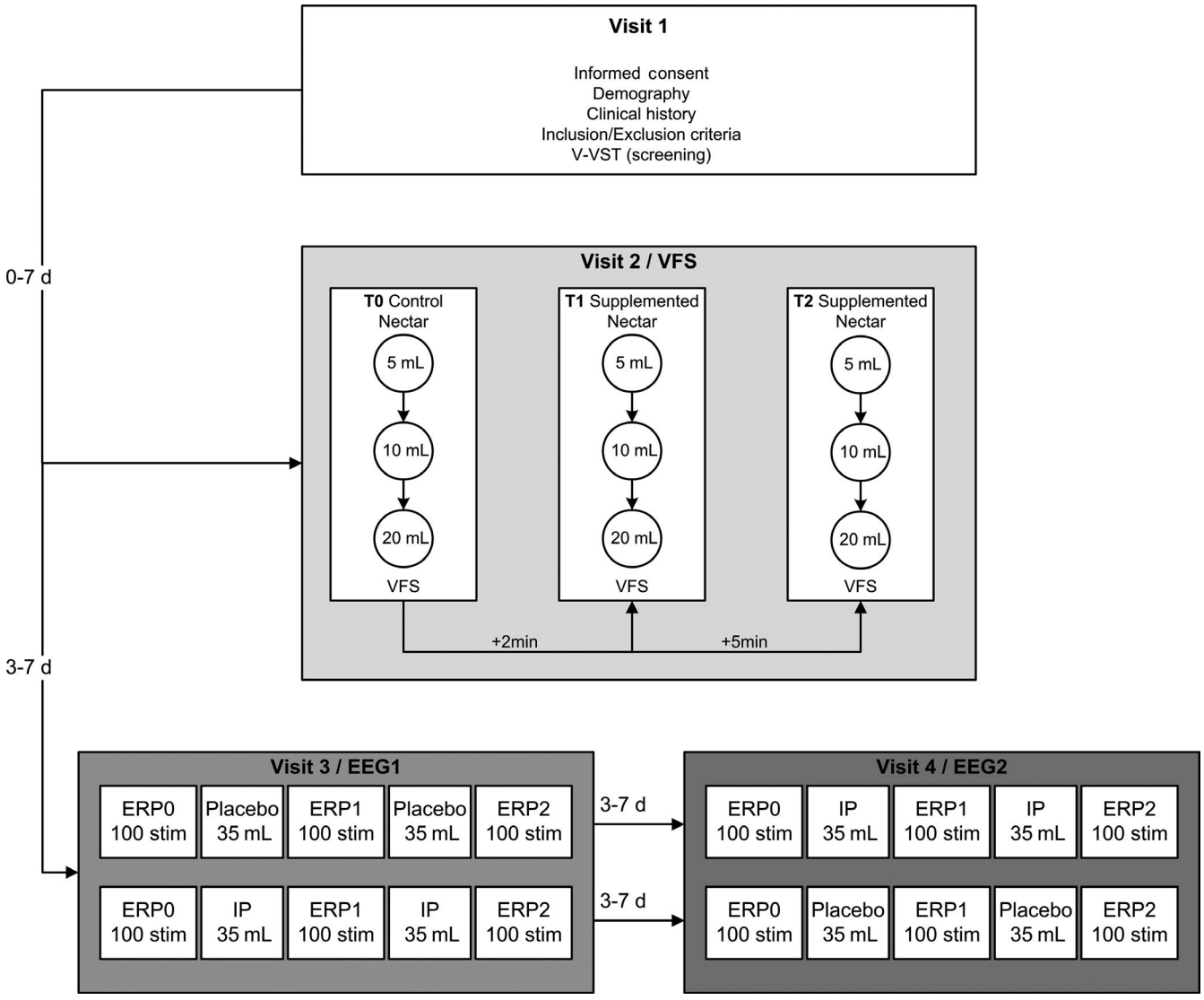

FIGURE 1 Study design including screening (Visit 1), videofluoroscopy (Visit 2), and EEG (Visits 3 and 4). Note that during VFS, each patient acts as his/her own control. EEG, electroencephalogram; ERP, evoked-related potential; IP, investigated product; VFS, videofluoroscopy

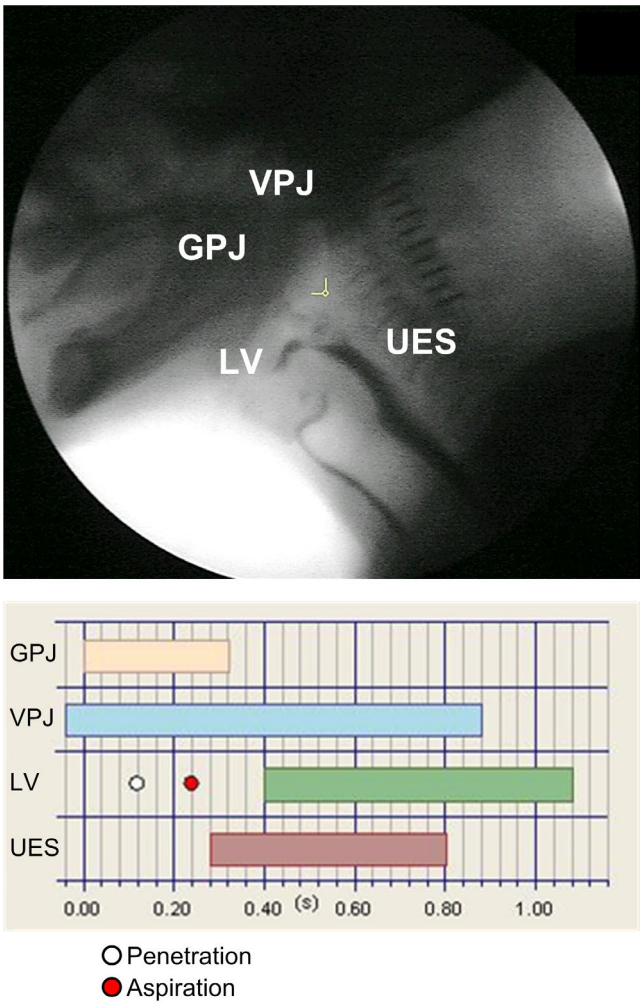

FIGURE 2 Chronogram of the oropharyngeal swallow response in a patient with an aspiration. The white point depicts time the bolus enters the laryngeal vestibule (penetration) and the red point the time the aspiration takes place. GPJ, glossopalatal junction; LV, laryngeal vestibule; UES, upper esophageal sphincter; VPJ, velopharyngeal junction

152 template,[44] with the three-dimensional solution space restricted to cortical gray matter, as determined by the probabilistic Talairach atlas.[45] The intracerebral volume is partitioned in 6239 voxels at 5 mm spatial resolution. Anatomical labels like Brodmann areas (BA) are also reported using standard stereotactic (MNI) space, with correction to Talairach space.[46] In order to simplify the representation of the changes found in the source localization map with sLORETA after each treatment (T0 vs T1 and T2), only the lateral projection of the left hemisphere is represented in the Figure S2.

3 | RESULTS

For this study, 191 patients were assessed with the V-VST and 104 fulfilled the inclusion criteria and were referred for a VFS. Following the results of the VFS, 58 patients were finally included in the study and randomization into treatment groups: 21 for cinnamaldehyde-zinc (CIN-Zn), 21 for citral (CIT), and 16 for citral/isopulegol (CIT-ISO) (Figure S1).

3.1 | Demographics and OD etiology

Patients included in the study had a mean age of 79.8 ± 7.6 years, were a similar number of men and women (55.2% women), and presented a high number of co-morbidities (Charlson index) and mildly impaired functional status according to the Barthel index, without differences between groups (Table 1). The main cause of OD for the included patients was aging (56.9%) followed by neurodegenerative diseases (22.4%) and chronic stroke (20.7%) also without differences between groups ($P = .963$).

3.2 | Baseline VFS signs and swallow biomechanics

All patients had VFS signs of impaired safety of swallow (PAS ≥ 2) with penetrations into the laryngeal vestibule or aspirations at nectar control series; the mean PAS among patients was 3.86 ± 1.43 and up to 8.62% patients presented aspirations. Regarding swallowing efficacy, 70.7% of the patients had oral residue and 56.9%, pharyngeal residue at nectar control series. Swallow response was also severely impaired in all patients, with delayed time to LVC when compared with normative data from healthy volunteers (HV) studied by our group under the same experimental conditions (398.6 ± 151.2 ms in patients vs 174.3 ± 11.6 ms in HV)[12] and time to UESO (287.6 ± 114.7 ms in patients vs 234.3 ± 8.2 ms in HV at the same viscosity of the same thickening agent)[12] (Table 2). The basal kinetic measures (mean bolus velocity and propulsion force) of a 5 mL nectar bolus were similarly impaired between groups (Table 2).

There were no learning effects observed between the fixed sequential administration of boluses. PAS score and time to LVC worsened between the three control boluses which would not have been the case if there had been a learning effect: mean PAS was 2.3 ± 1.7 in 5 mL, 2.6 ± 1.7 in 10, and 3.3 ± 1.4 in 20 mL (5 mL vs 20 mL: $P < .0001$; 10 mL vs 20 mL: $P = .019$); and mean LVC was 398.6 ± 151.1 ms in 5 mL, 416.3 ± 126.9 ms in 10 mL, and 497.4 ± 166.9 ms in 20 mL (5 mL vs 10 mL: $P = .001$; 10 mL vs 20 mL: $P = .001$).

3.3 | Baseline neurophysiology

Pharyngeal sensory function was also impaired in the study participants. Patients had high sensory threshold to pharyngeal electrical stimuli (10.41 ± 4.93 mA in patients vs 6.0 mA in HV),[18] while the mean tolerance threshold was also higher than the one previously found in HV (29.14 ± 12.96 mA in patients vs 24 mA in HV).[18] The pharyngeal ERP wave form in this study was

characterized by two negative (N1, N2) and two positive peaks (P1, P2) as we previously described in similar patients with OD.[18] The ERP was also similarly impaired in our patients: the N2 peak latency was longer (228.27 ± 46.60 ms in patients vs 190 ms in young HV),[18] and the N1-P1 and N2-P2 amplitudes were lower (3.20 ± 2.21 μV and 3.69 ± 2.99 μV in patients vs 6.6 μV and 10.9 μV in young HV).[18]

3.4 | Effect of treatments on VFS signs and swallow biomechanics

Only the administration of CIN-Zn reduced the prevalence of signs of impaired swallowing safety during VFS. The PAS score was significantly reduced in the CIN-Zn group during the second administration (T2) of the IP (4.00 ± 1.48 vs 3.19 ± 1.81, P = .009) (Figure 3), and the prevalence of patients with safe swallows increased from 52.38% to 80.95% at T1 (P = .041). None of the TRP agonists significantly affected the prevalence of oral residues (from 70.7% to 81.0%, ns) or pharyngeal residues (from 56.9% to 67.2%, ns).

The administration of both CIN-Zn and CIT significantly reduced the timing of oropharyngeal reconfiguration from a respiratory to a digestive pathway by shortening time to LVC and to UESO. Both series of the CIN-Zn group significantly reduced time to LVC (401.9 ± 168.0 ms vs 330.0 ± 124.4 ms, P = .008 during T1 and vs 336.0 ± 144.2 ms, P = .002 during T2) and time to UESO (291.4 ± 138.1 ms vs 238.0 ± 98.4 ms, P = .023 during T1 and vs 238.0 ± 132.0 ms, P = .007 during T2). In the CIT group, only the first administration significantly reduced time to LVC (457.1 ± 155.7 ms vs 405.7 ± 117.5 ms, P = .023) and time to UESO (310.5 ± 113.8 ms vs 272.4 ± 88.2 ms, P = .035). CIT-ISO significantly reduced time to UESO during both administrations (252.5 ± 72.6 ms vs 215.0 ± 78.5 ms, P = .038 during T1 and vs 218.7 ± 78.4 ms, P = .017 during T2) (Figure 4).

TABLE 1 Socio-demographic and clinical characteristics of the population in the three arms of the study

	Total	CIN-Zn	CIT	CIT-ISO	*P*-value
N	58	21	21	16	
Older (%)	56.9	61.9	57.1	50	.769
Neurodegenerative disease (%)	22.4	19.0	23.8	25	.895
Stroke (%)	20.7	19.0	19.0	25	.882
Age (y)	79.8 ± 7.6	81.6 ± 7.6	78.0 ± 7.5	79.8 ± 7.9	.233
Sex (% women)	55.2	61.9	57.1	43.6	.532
Barthel index	66.6 ± 33.4	75.8 ± 33.8	58.5 ± 30.7	63.2 ± 35.6	.444
Charlson index	2.8 ± 1.6	2.8 ± 1.3	3 ± 2.0	2.6 ± 1.6	.792

Note: Data are presented as mean ± SD unless specifically stated.

Abbreviations: CIN-Zn, Cinnamaldehyde-zinc treatment group; CIT, Citral treatment group; CIT-ISO, Citral-isopulegol treatment group.

TABLE 2 Swallow biomechanics characteristics of the study participants

	Total (n = 58)	CIN-Zn (n = 21)	CIT (n = 21)	CIT-ISO (n = 16)	*P*-value
PAS	3.86 ± 1.43	4.00 ± 1.48	3.90 ± 1.26	3.63 ± 1.63	.557
Oral residue	41 (70.7%)	14 (66.7%)	15 (71.4%)	12 (75.0%)	.855
Pharyngeal residue	33 (56.9%)	13 (61.9%)	10 (47.6%)	10 (62.5%)	.561
LVC (ms)	398.6 ± 151.2	401.9 ± 168.0	457.1 ± 155.7	317.5 ± 70.8	.049
UESO (ms)	287.6 ± 114.7	291.4 ± 138.1	310.5 ± 113.8	252.5 ± 72.6	.243
LVO (ms)	1121 ± 333.9	1105 ± 255.0	1141 ± 431.5	1118 ± 296.3	.998
Mean velocity (m/s)	0.24 ± 0.14	0.25 ± 0.14	0.24 ± 0.18	0.24 ± 0.09	.586
Propulsion force (mN)	307.6 ± 425.3	338.9 ± 425.7	323.6 ± 561.0	245.4 ± 142.4	.759

Note: Data presented as mean ± SD except for oral and pharyngeal residue (%).

Abbreviations: CIN-Zn, Cinnamaldehyde-Zinc group; CIT, Citral group; CIT-ISO, Citral-Isopulegol group; LVC, laryngeal vestibule closure; PAS, penetration-aspiration scale; UESO, upper esophageal sphincter opening.

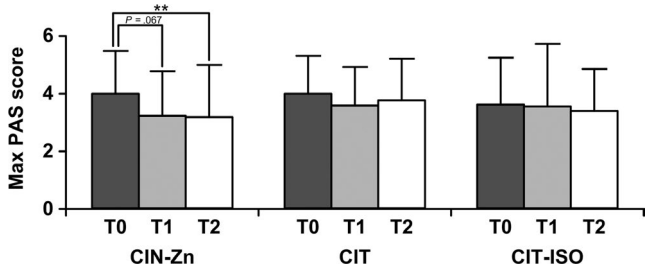

FIGURE 3 Representation of the mean ± SD maximum PAS score registered during the V2 VFS. CIN-Zn, Cinnamaldehyde-zinc group; CIT, Citral group; CIT-ISO, Citral-isopulegol group; T0, control series; T1, first administration; T2, second administration. **P*-value < .01

3.5 | Effect of treatments on swallow neurophysiology

3.5.1 | Pharyngeal sensory evoked potentials

Baseline data on the ERP were similar among the 3 groups (T0; *P* > .05 for all comparisons). The administration of CIN-Zn significantly reduced the latency of the P2 peak and the amplitude of the N2-P2 peaks of the ERP; moreover, this effect was significantly greater than the small changes caused by placebo in these patients. Both ERP registered after CIN-Zn administration presented a shorter P2 latency compared with the baseline (374.0 ± 45.0 ms vs 350.2 ± 44.7 ms, *P* = .050 in T1 and 332.8 ± 44.1 ms, *P* = .005 in T2). The ERP registered after the second CIN-Zn administration presented a significantly reduced amplitude of the N2-P2 peaks compared with the baseline ERP (5.88 ± 4.40 mA vs 3.74 ± 2.45 mA, *P* = .005); this effect was not observed when administering placebo to the same patients.

Among the other peaks and treatment groups, only the ERP registered after the second CIT administration presented a significantly reduced amplitude of the P1-N2 peaks (2.93 ± 2.57 mA vs 2.48 ± 2.64 mA, *P* = .042) (Figure 5).

3.5.2 | Neurotopography

At T0, the source cortical activation areas through sLORETA analysis (Figure S2) agreed with our previously reported studies on older patients with OD,[18] showing bi-hemispheric activation of prefrontal and anterior temporal cortices in early peaks (N1 and P1) and activation of parietal and posterior cingulate cortices in late peaks (N2 and P2).

Temporary significant changes on source cortical activation occurred at T1 (with respect to T0) with all three treatments (Figure 6; detailed description in the Appendix S1) but none of them remained at T2: Increased activation was shown in frontal gyri (Cin-Zn, CIT, and CIT-ISO), precuneus (CIT), and cingulate gyrus (CIT-ISO), and decreased activation was shown in inferior frontal gyrus (Cin-Zn),

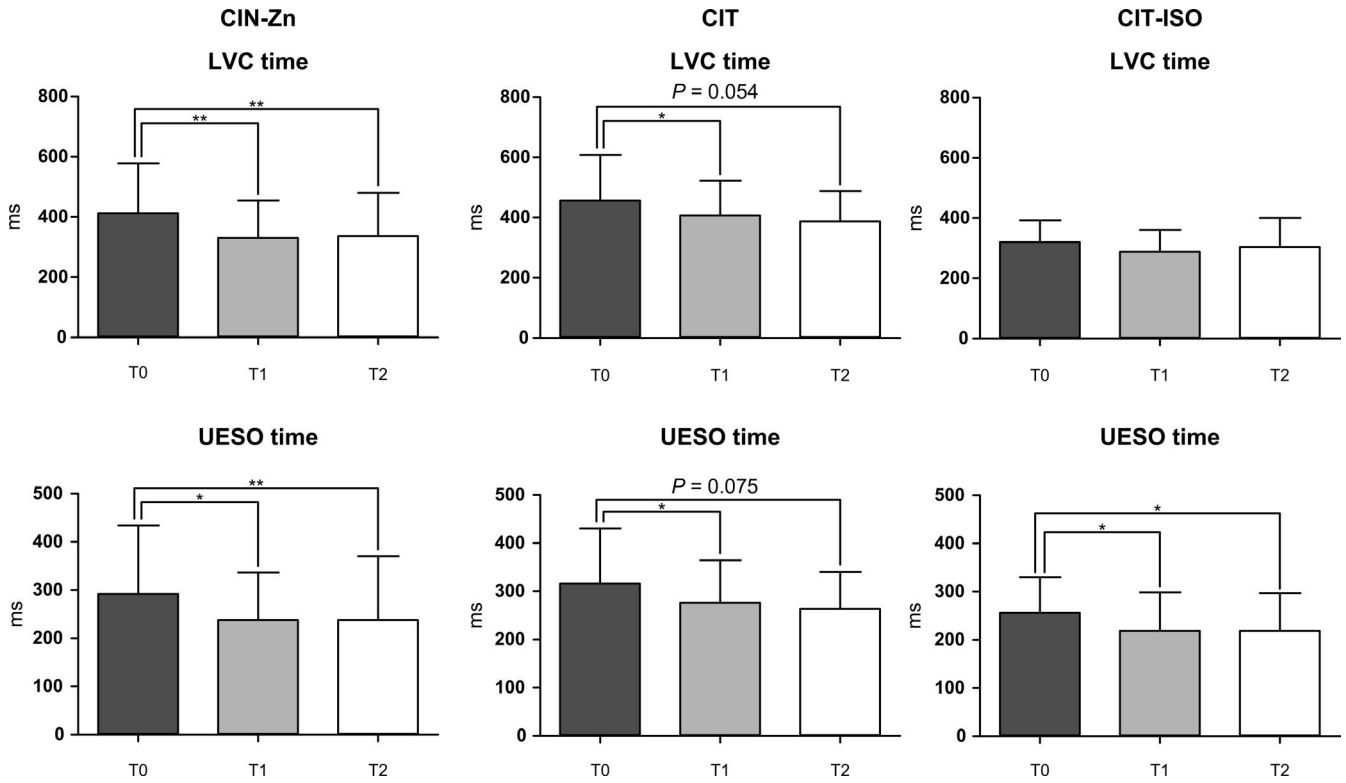

FIGURE 4 Effect of TRP agonists on the mean ± SD timing of the swallow response: LVC and UESO. CIN-Zn, Cinnamaldehyde-Zinc group; CIT, Citral group; CIT-ISO, Citral-Isopulegol group; LVC, laryngeal vestibule closure; T0, control series; T1, first administration; T2, second administration; UESO, upper esophageal sphincter opening. **P*-value < .05; ***P*-value < .01

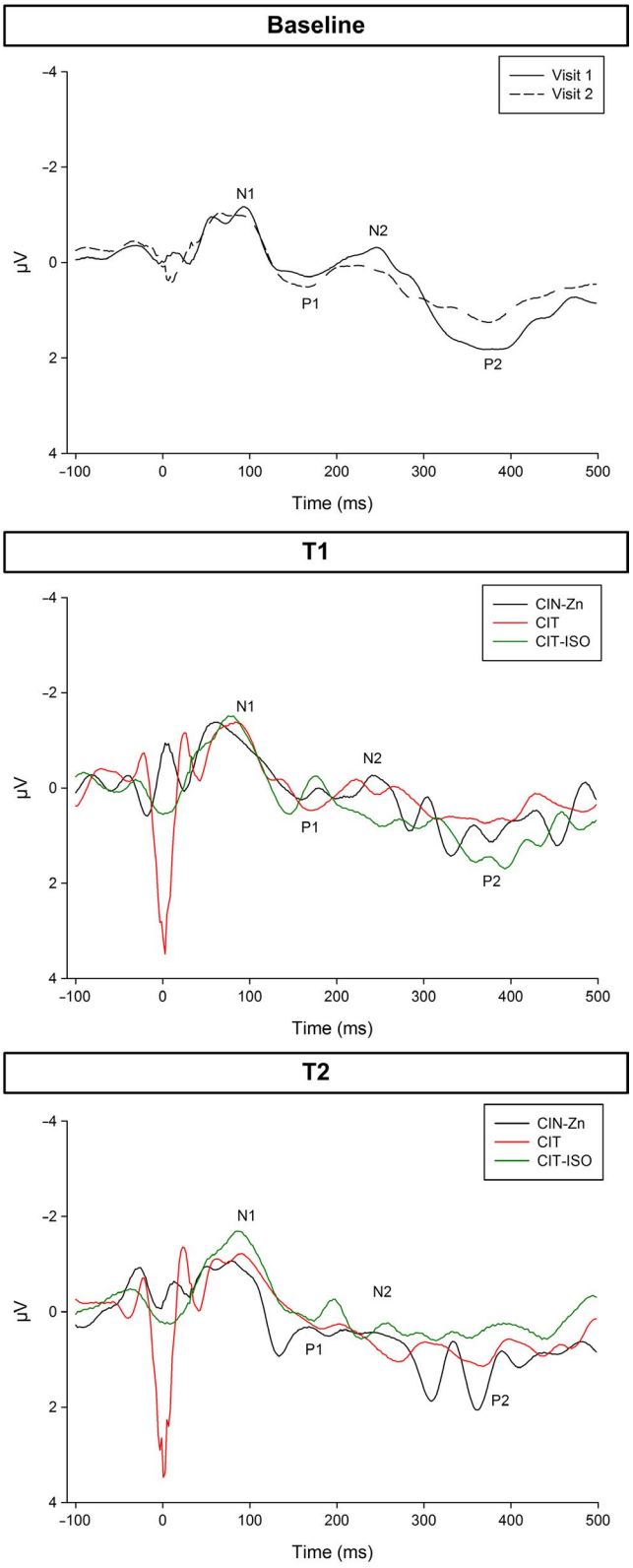

FIGURE 5 Pharyngeal event-related potential (ERP) traces obtained at Cz for each treatment at baseline, T1 and T2 after pharyngeal electrical stimulation. Deflection at time point 0 corresponds to stimulus artifact. μV, microvolts; CIN-Zn, Cinnamaldehyde-zinc group; CIT, Citral group; CIT-ISO, Citral-isopulegol group; T1, first administration; T2, second administration

postcentral gyrus (CIT), and precuneus (CIT-ISO). Persistent significant T2 changes (with respect to T0) in the source cortical activation showed as significant focal enhancements only for the transverse temporal gyrus with Cin-Zn (N1-peak) and for the superior frontal gyrus and insula with CIT-ISO (P1 and N2-peaks) represented by deep blue color in the T0 vs T2 panel, while no significant increased activation was found with CIT (Figure 6, Figure S2).

3.6 | Palatability of the treatments

Patients rated the IPs on a 0-10 scale as mildly intense compared with the placebo, although only the CIT group achieved significance (2.62 ± 2.48 with placebo vs 5.50 ± 2.84 with CIT, $P = .002$). Regarding pleasantness, only CIT administration was perceived as less pleasant than placebo, but this difference was not significant (5.38 ± 2.77 with placebo vs 4.35 ± 2.76 with CIT, $P = .057$). The treatment with the lowest prevalence of registered gut discomfort was CIN-Zn but there were too few to find significant differences between treatments.

3.7 | Safety of the treatment

During the study, twelve patients reported minor adverse events of six kinds. No relation was found between these and the IP except for pharyngeal irritation declared as possibly related to CIT-ISO. The most frequent event (75%) was gastrointestinal disorders (diarrhea and vomiting) related to the contrast agent used during VFS. No severe adverse events were reported.

4 | DISCUSSION

The main results of this study are that supplementation of the nectar bolus with cinnamaldehyde and zinc (CIN-Zn, TRPA1 agonists) significantly improved swallowing safety and the neurophysiology and biomechanics of the swallow response in our patients. The study also shows that CIN-Zn is safe, palatable, and well-tolerated. On the other hand, citral (TRPA1 agonist) and the combination of citral and isopulegol (TRPA1/M8 agonists) slightly improved the swallow response but had no significant effect on the VFS signs of safety impairments of OD. These results suggest the benefits of using the TRPA1 agonist CIN-Zn (756.6 μmol/L cinnamaldehyde and 70 μmol/L zinc) as an active treatment for OD when combined with a xanthan gum thickener at 182 ± 2 mPa·s viscosity.

Prevalence of malnutrition, co-morbidities, and polypharmacy with potential effects on swallowing function among the study populations was very high, putting patients at risk of developing further serious respiratory—aspiration pneumonia—and nutritional complications, readmissions and mortality.[10,47,48] Our restrictive inclusion criteria were designed to homogenize the sample as much

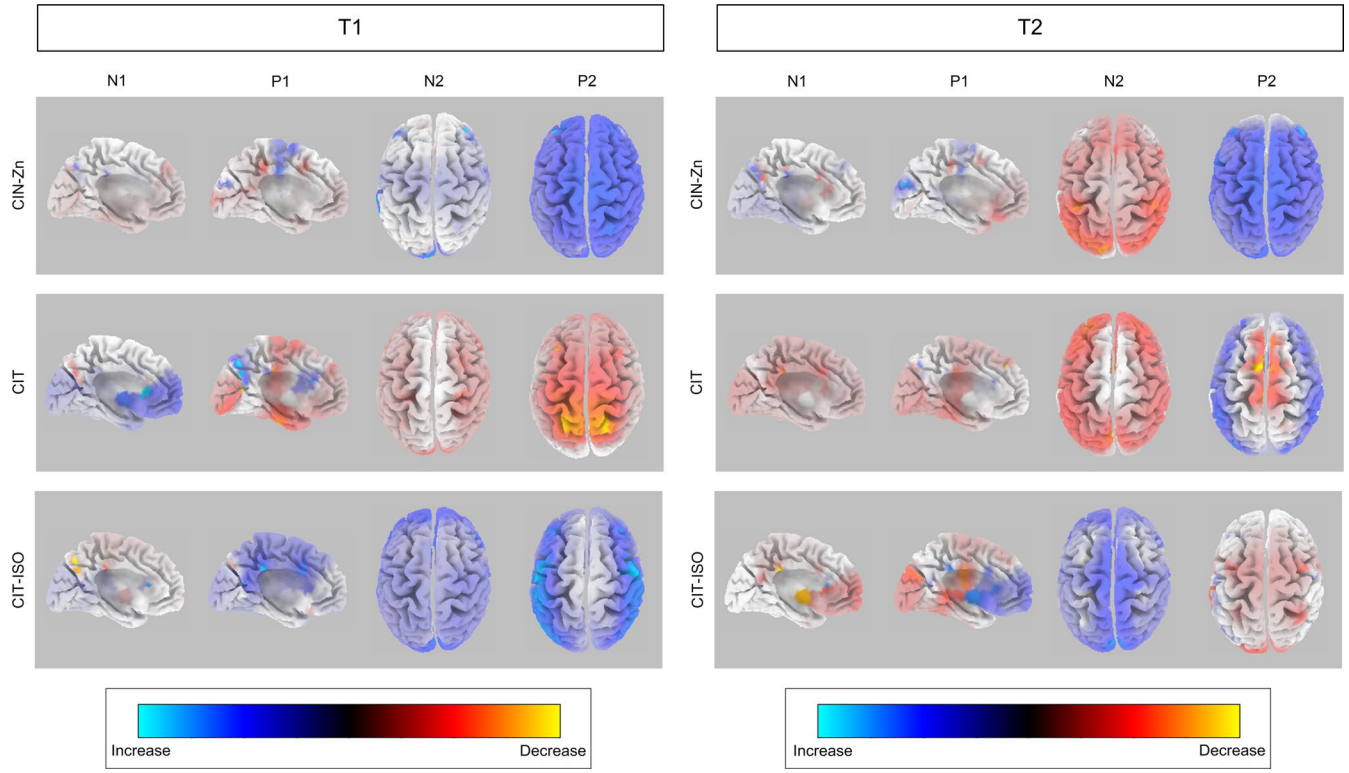

FIGURE 6 sLORETA comparison between baseline and T1 and T2 of each group. Note the increased activation in frontal gyri (CIN-Zn, CIT, and CIT-ISO) and precuneus (CIT) after the first application of the treatments and the enhanced activation of the transverse temporal gyrus (CIN-Zn) after the second. CIN-Zn, cinnamaldehyde-zinc groups; CIT, Citral group; CIT-ISO: citral-isopulegol group; T1, first administration; T2, second administration

as possible to investigate the immediate effect of TRP agonists on swallow biomechanics and neurophysiology in patients receiving thickening agents as compensatory treatment. The high dropout rate observed after the VFS in our study was caused by the inclusion criteria which required a positive V-VST during the deglutition of any volume and viscosity level, whereas the randomization criteria were to have a PAS > 2 during the 5 mL nectar bolus in the VFS study. This study further confirms the severe impairment of the biomechanics of the OSR and pharyngeal sensory function in patients with OD associated with aging and neurological diseases.VFS signs of impaired safety were related to a delayed swallow response, more precisely to a delayed time to laryngeal vestibule closure (LVC), as found in our previous studies.[12,49]

Time to LVC is a critical biomechanical event, relevant to the occurrence of penetrations and aspirations into the airway.[12,49] The results of this study and our previous studies suggest that reducing time to LVC and improving bolus propulsion and velocity are key targets for active treatments aiming at improving the swallow response in patients with OD.[49]

OD was also associated with impaired oropharyngeal sensory perception in this study. In previous studies of our group, we found that older HV and older OD patients had significantly higher sensory thresholds than young HV.[18] The decrease of pharyngeal sensitivity with age had been also described by Aviv et al[16] and related to neurodegenerative peripheral nerve impairments.[50] The patients included in this study also had a high pharyngeal sensory threshold to electrical stimuli. In addition, they had longer N2 latency and lower N1-P1 and N2-P2 amplitudes than HV, agreeing with our previous studies.[18] The sLORETA analysis showed that the cortical source localization of sensory perception in our patients was mainly focalized in areas of the frontal lobe and the cingulate cortex.

In the present study, mean PAS score with nectar viscosity was almost 4 and CIN-Zn reduced PAS from 4.00 ± 1.48 at T0 with the nectar control series to 3.19 ± 1.81. Nectar supplemented with any of the treatments assayed in this study improved the OSR times of the patients, but only CIN-Zn significantly improved swallow safety. The stimulation of TRPA1 with CIN-Zn significantly reduced the PAS score and the prevalence of laryngeal penetrations by significantly shortening time to LVC, similar to the effect we found with piperine, an agonist that activates both TRPV1 and TRPA1, in an earlier study.[35,51] Furthermore, CIN-Zn significantly reduced the time to UESO, an effect we found with capsaicinoids.[35] In our study, CIT (TRPA1 agonist) improved both the time to LVC and to UESO, and prevalence of safe swallows but in contrast CIT-ISO (TRPA1/TRPM8 agonists) only had a significant effect on the time to LVC. A cross-desensitization effect between cinnamaldehyde (TRPA1 agonist) and menthol (TRPM8 agonist) has been described in rat trigeminal subnucleus caudalis neurons

and could be the cause of the difference between the effect of our TRPM8 and TRPA1 agonists.[52] In addition, we observed significant neurophysiological improvements with patients who received CIN-Zn treatment who showed a shorter P2 peak latency in comparison with placebo, after the first (−23.8 ms) and the second (−41.2 ms) administration, and a reduction in the N2-P2 amplitude in the ERP2 (−10.66 ± 9.64% of change, P = .049). These neurophysiologic improvements could be related to the biomechanical improvements observed, and these effects on brain plasticity should be taken into account in future studies.

The TRP agonist concentrations assayed in this study were similar to those used in flavorings and were perceived as pleasant and were well-tolerated by OD patients who did not show signs of gut discomfort. Only the CIT IP was perceived as significantly more intense and less pleasant compared to placebo. Finally, studies from our group using a TRPV1 agonist (capsaicinoids) therapy over 10 days showed improvements in both biomechanical and neurophysiological swallowing responses without any desensitization processes.[53,54] This suggests that chronic treatments with TRP agonists could be useful as an active treatment for OD but more studies are needed to evaluate the long-term effect of this promising therapy.

This study has some limitations. The first one is that we included three OD patient phenotypes in the study (elderly, neurodegenerative disease, stroke); further studies with larger sample sizes of each phenotype are needed in order to make sub-analyses comparing the effect of the treatment between phenotypes. In addition, we have evaluated the acute effect (single dose) of the TRPA1 and TRPM8 agonists. Studies describing the long-term effect of repetitive doses of these agonists will clarify whether the observed biomechanical and neurophysiological changes are maintained over time. Finally, whereas the EEG studies had a placebo arm in a crossover design, the VFS studies did not have a separate control group to avoid unnecessary exposure to radiation. However, the improvements observed in biomechanics after the treatment with the TRP agonists cannot be explained by learning effect although the order of exposition was always the same. We did not find any improvement at T0 when compared the bolus of 5, 10, and 20 mL during VFS, allowing us to attribute the improvement to the TRP agonists.

In summary, TRPA1 acute stimulation with CIN-Zn or CIT improves swallow response which, in the case of CIN-Zn, is associated with a significant modification in cortical activation and improves swallowing safety. These results are foundational for the development of new active pharmacological treatments for OD by combining TRPA1 agonists and a xanthan gum thickener. This is a relevant advance in the new paradigm of treatments for patients with OD, from compensation to the recovery of swallow function.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

PC, LR, CL, and SM designed the research study; NT, LR, OO, LM, and DA performed the research; LR, NT, CC, and DA analyzed the data; VA, WN, and AM performed and analyzed VFS studies; NT, DA, and MB wrote the article. All the authors reviewed the article and approved the final version.

ORCID

Noemí Tomsen https://orcid.org/0000-0001-7149-7948
Daniel Alvarez-Berdugo https://orcid.org/0000-0003-0338-0793
Laia Rofes https://orcid.org/0000-0002-1628-5253
Omar Ortega https://orcid.org/0000-0001-6178-3748
Viridiana Arreola https://orcid.org/0000-0003-3913-317X
Weslania Nascimento https://orcid.org/0000-0003-1267-3475
Alberto Martin https://orcid.org/0000-0002-6805-764X
Christopher Cabib https://orcid.org/0000-0002-1876-6923
Mireia Bolivar-Prados https://orcid.org/0000-0002-8896-1150
Lluís Mundet https://orcid.org/0000-0001-9091-8998
Pere Clavé https://orcid.org/0000-0002-0696-8560

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Effect of Aging, Gender and Sensory Stimulation of TRPV1 Receptors with Capsaicin on Spontaneous Swallowing Frequency in Patients with Oropharyngeal Dysphagia: A Proof-of-Concept Study

Weslania Nascimento [1,†], Noemí Tomsen [1,2,3,†], Saray Acedo [4], Cristina Campos-Alcantara [4], Christopher Cabib [2], Marta Alvarez-Larruy [1,5] and Pere Clavé [1,2,*]

1 Gastrointestinal Physiology Laboratory, Department of Surgery, Hospital de Mataró, Universitat Autónoma de Barcelona, 08304 Mataró, Spain; wdo@csdm.cat (W.N.); ntomsen@csdm.cat (N.T.); malvarezla@csdm.cat (M.A.-L.)
2 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, 28029 Madrid, Spain; ccabibitalia@yahoo.it
3 Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
4 Facultat de Biologia, University of Barcelona, 08028 Barcelona, Spain; acedosaray@gmail.com (S.A.); cristinacamposub@gmail.com (C.C.-A.)
5 Department of Neurology, Hospital de Mataró, 08304 Mataró, Spain
* Correspondence: pere.clave@ciberehd.org; Tel.: +34-93-741-77-00
† These co-authors contributed equally to the study.

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Abstract: Spontaneous swallowing contributes to airway protection and depends on the activation of brainstem reflex circuits in the central pattern generator (CPG). We studied the effect of age and gender on spontaneous swallowing frequency (SSF) in healthy volunteers and assessed basal SSF and TRPV1 stimulation effect on SSF in patients with post-stroke oropharyngeal dysphagia (OD). The effect of age and gender on SSF was examined on 141 healthy adult volunteers (HV) divided into three groups: GI—18–39 yr, GII—40–59 yr, and GIII—>60 yr. OD was assessed by the Volume–Viscosity Swallowing Test (VVST). The effect of sensory stimulation with capsaicin 10^{-5} M (TRPV1 agonist) was evaluated in 17 patients with post-stroke OD, using the SSF. SSF was recorded in all participants during 10 min using surface electromyography (sEMG) of the suprahyoid muscles and an omnidirectional accelerometer placed over the cricothyroid cartilage. SSF was significantly reduced in GII (0.73 ± 0.50 swallows/min; $p = 0.0385$) and GIII (0.50 ± 0.31 swallows/min; $p < 0.0001$) compared to GI (1.03 ± 0.62 swallows/min), and there was a moderate significant correlation between age and SFF ($r = -0.3810$; $p < 0.0001$). No effect of gender on SSF was observed. Capsaicin caused a strong and significant increase in SSF after the TRPV1 stimulation when comparing to basal condition (pre-capsaicin: 0.41 ± 0.32 swallows/min vs post-capsaicin: 0.81 ± 0.51 swallow/min; $p = 0.0003$). OD in patients with post-stroke OD and acute stimulation with TRPV1 agonists caused a significant increase in SSF, further suggesting the potential role of pharmacological stimulation of sensory pathways as a therapeutic strategy for CPG activation in patients with OD.

Keywords: disorders; spontaneous swallowing frequency; capsaicin; TRPV Cation Channels; stroke; aging

1. Introduction

Spontaneous swallowing contributes to airway protection and a reduced spontaneous swallowing frequency (SSF) can lead to increased pharyngeal secretions and aspiration [1]. The neurophysiology of spontaneous swallows falls within the normal functionality of a brainstem reflex arc situated in the central pattern generator (CPG). The activity of CPG can be modified by inputs from cortical and subcortical areas of the brain [2,3]. Previous studies

with functional magnetic resonance imaging have shown that spontaneous saliva and water swallows activate the precentral motor and post-central somatosensory cortical areas [4,5], suggesting that the neural control of swallowing not only occurs in the brainstem but also in the cortex [6]. Under physiological circumstances, brainstem reflex circuits may receive excitatory or inhibitory descending nerve impulses from supratentorial structures that modulate final motor response. Excitatory supranuclear influences are proportional to brain excitability. This has been well documented in neurophysiological studies on the blink reflexes in patients with stroke or multiple sclerosis [7,8], and it is hypothesized that the nerve control of spontaneous swallowing could be similar. The SSF in healthy volunteers is 1.32 swallows/min [9], which decreases to approximately 0.6 swallows/min in older people without swallowing impairments [10]. Patients with acute stroke and oropharyngeal dysphagia (OD) showed a significant reduction in SSF (0.23 swallows/min) compared to post-stroke patients without OD (0.56 swallows/min), suggesting that a SSF $\leq$ 0.40 swallows/min could indicate swallowing impairments [11,12] and identify dysphagia with psychometric properties equal or superior to clinical screening protocols [12]. In addition, a correlation between SSF and salivary substance P (SP) concentration has been described; a low concentration of SP in saliva predicts a significant reduction in SFF and a higher incidence of pneumonia in patients with acute stroke [13].

The human oropharynx is highly innervated by the sensory branches of the cranial nerves V, VII, IX, and X [14]. The TRPV1 receptor has been located in the plasma membrane of the epithelial cells and nociceptive Aδ fibers located in the submucosa near the basal lamina of the human oropharyngeal mucosa [15]. This receptor can be activated with several endogenous and exogenous stimuli, such as pH and temperature changes and natural agonists like capsaicin, facilitating the transmission of sensory inputs through the afferent pathway by the release of several neuropeptides such as SP and CGRP. The role of these neuropeptides in swallowing is still unknown, but it is hypothesized that it could be similar to that described for the cough reflex. In this case, the neuropeptide SP sensitizes the mechanoreceptor fibers and improves the transmission of sensory inputs from the peripheral nerves to the brainstem [16]. In addition, our group found that those patients with OD as a consequence of aging or stroke had lengthened latency and reduced amplitude of the pharyngeal sensory evoked potentials (PSEP) characteristic peaks, in addition to a loss of symmetry of the PSEPs and their cortical representation in patients with chronic post-stroke dysphagia. Our results suggest that impaired conduction and integration of the sensory inputs could affect the efferent pathway, leading to an impaired oropharyngeal swallow response [17,18].

Several studies have demonstrated the therapeutic effect of capsaicin on the biomechanics and neurophysiology of the swallow response in patients with OD. Acute (or single dose) stimulation with capsaicin at 150 μM reduces the prevalence of safety and efficacy impairments of swallowing, and strongly improves the time to laryngeal vestibule closure (LVC) and to upper esophageal sphincter opening (UESO) [19,20]. Similar results have been observed when patients were treated three times a day during 10 days with a lower dose (10 μM). In this case, not only a reduction in time to LVC and PAS score were found, but also a faster and more intense neurophysiological response and significant changes in brain activation, as well as a positive correlation between the variation of N1 peak latency of PSEPs and improved time to LVC [21,22]. Regarding the effect on neuropeptide secretion, an increase in the concentration of salivary SP and CGRP was observed after TRPV1 stimulation with capsaicin [23,24]. Taken all together, these findings suggest that the TRPV1 agonist capsaicin could be used as a potential treatment to improve SSF.

The aims of this proof-of-concept study are (1) to assess the effect of age and gender on SSF in healthy volunteers, (2) to examine whether patients with post-stroke OD have lower SSF, and (3) to assess the effect of TRPV1 stimulation with capsaicin on SSF in patients with post-stroke OD.

2. Materials and Methods

2.1. Study Population

This study has two branches: (1) an observational one-day study to assess the effect of age and sex on spontaneous swallow frequency (SSF) in healthy volunteers and (2) an interventional study to assess the effect of TRPV1 stimulation with capsaicin at 10 µM on SSF in post-stroke dysphagia (PSD) patients. The study protocol was approved by the Ethical Committee of the Hospital de Mataró (11/17) and performed according to the rules of the Declaration of Helsinki. All the participants signed the informed consent.

2.1.1. Healthy Volunteers

A total of 141 healthy volunteers (18–90 years old) were recruited from the community and divided in three groups according to age: 18–39 years (GI), 40–59 years (GII), and ≥60 years (GIII). The inclusion criteria were to be ≥18 years old without previous diagnosis of swallowing disorders or using any medication that could influence the saliva flow (such as antidepressants, antipsychotics, or anticholinergics). The exclusion criteria were head and neck surgery/radiotherapy or neurological disorders including neurodegenerative diseases.

2.1.2. Stroke Patients

Seventeen stroke patients were recruited through the Neurology Department of the Hospital de Mataró. All patients had signs of impaired swallowing safety according to the Volume–Viscosity Swallowing Test [25]. The exclusion criteria were to have a life expectancy of less than three months, OD diagnosis previous to the stroke episode, or OD associated a pathology other than stroke.

2.2. Experimental Design

For the observational study, SSF was measured during 10 min in HV and PSD patients. All participants were requested to avoid body and head movements and talking during the experiment. For the study on the effect of capsaicin on SSF, PSD patients received TRPV1 stimulation treatment using a 10 µM capsaicin solution administered orally (four bolus of 5 mL) after the first 10-min SSF recording. In the intervals between capsaicin intakes, patients were requested to make dry swallows (two to four). A second 10-min SSF recording was made following the capsaicin treatment (Figure 1).

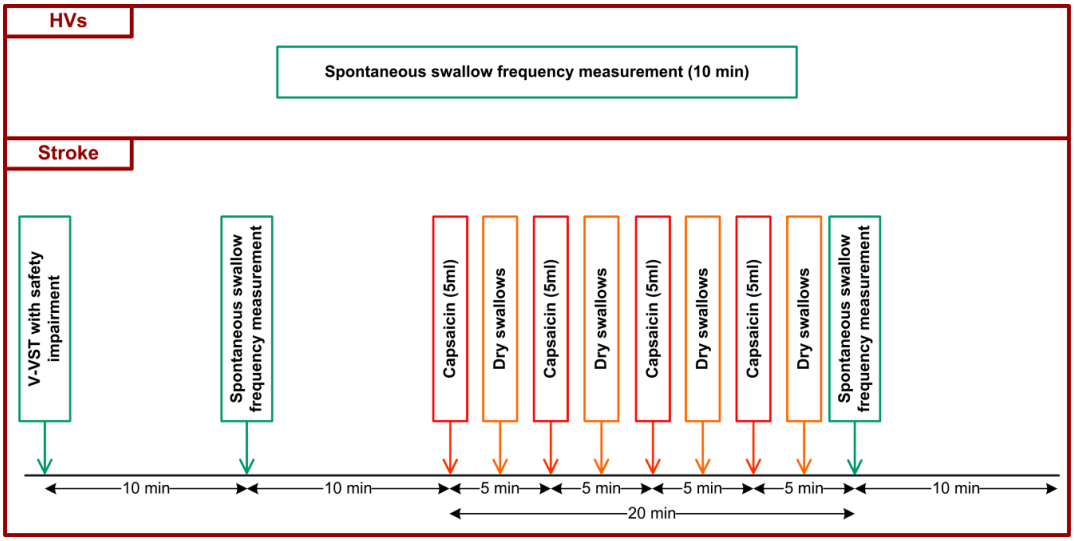

Figure 1. Study design of the two populations included in the study: HV at the top, post-stroke patients at the bottom.

2.3. Volume–Viscosity Swallowing Test (V-VST)

Stroke patients were screened with the V-VST, as previously described [25]. The swallowing function was assessed while swallowing boluses of 5, 10, or 20 mL of liquid (<50 mPa·s), nectar (98.61 ± 3.78 mPa·s), or pudding (4539.50 ± 530.93 mPa·s). Nectar and pudding were obtained by adding 4.5 and 9 g of Resource ThickenUp (Nestle, Barcelona, Spain), respectively, to 100 mL of water. Only those patients that presented signs of safety impairment (cough, voice changes, and/or a decrease of ≥3% of oxygen saturation) were included.

2.4. Spontaneous Swallow Frequency Recordings (SSF)

SSF was measured with surface neck electromyography (EMG) and accelerometry. After cleaning the skin with alcohol, EMG electrodes were positioned over the suprahyoid muscles, while an omnidirectional accelerometer was placed over the cricothyroid cartilage (Supplementary Figure S1). This omnidirectional accelerometer assesses acceleration in multiple directions, but is more sensitive to movement in the vertical plane (Puyau, 2004). SSF was recorded for 10 min. All recordings were analyzed offline using the AcqKnowledge software (BIOPAC Systems Inc., Goleta, CA, USA), which displays a visual trace of the EMG, the complete EMG, and the accelerometer signal. A spontaneous swallow was considered when the signal was registered by both EMG and the accelerometer (Supplementary Figure S2). The spontaneous swallowing rate was calculated as spontaneous swallows per minute. In addition, the following EMG metrics were analyzed for each swallow: amplitude (distance from highest to lowest peak of the EMG signal expressed in volts), duration, delta T (ΔT, time from start to end in seconds), and area under the curve (AUC, amplitude and ΔT integral, in volts·seconds).

2.5. Intervention: Effect of Oropharyngeal Sensory Stimulation with TRPV1 Agonists

To evaluate the effect of TRPV1 stimulation on SSF, participants were given four 5 mL boluses of 10^{-5} M capsaicin (Spectrum chemical MFG Corp, New Brunswick, NJ, USA), 5 min apart. Between each bolus, patients were requested to perform 2–4 dry swallows depending on their tolerance.

2.6. Data Analysis and Statistical Methods

Continuous data were described as mean ± standard deviation (SD) and categorical data as absolute or relative frequencies. Continuous data were analyzed by one-way ANOVA and Dunn's multiple comparison post-test (age effect), unpaired *t*-test (gender × age effect), or paired *t*-test (pre vs. post TRPV1 stimulation). Categorical data were compared by Fisher's exact test. The correlation between age and SSF was determined with Spearman's correlation coefficient. A non-parametric test was performed when appropriate (non-Gaussian data). In order to know if the variables age and sex (independent variables) had a direct effect on SSF (dependent variable), a multiple linear regression test was performed. Statistically significant differences were considered when p-value < 0.05. Statistical analysis was performed using the software GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Study Population

A total of 141 healthy volunteers were included in the exploratory study and divided as follows: 50 in GI (28.08 ± 5.84 years, 54.00% women), 49 in GII (50.08 ± 4.71 years, 51.02% women), and 42 in GIII (63.38 ± 7.92 years, 47.62% women).

Regarding the PSD patients, a total of 17 PSD patients (74.94 ± 11.43 years, 41.2% women) were included in the study, 11 following an acute stroke and six patients with a chronic stroke (≥3 month from the stroke onset). Mean Rankin was f 3.35 ± 0.93, Barthel index was 69.41 ± 27.43, and NIHSS was 6.59 ± 5.23. The main type of stroke was ischemic (90.10%), and most of them were located in the left hemisphere (43.75%). According to

the Oxford classification, 18.75% had a total anterior circulation infarct, 43.75% partial anterior circulation infarct, 6.25% lacunar infarct, and 31.25% had posterior circulation infarct. V-VST results showed that 100% of PSD patients had signs of impaired safety of swallow and 82.35% also had efficacy impairment signs. Liquid was the most unsafe viscosity (70.59%), followed by nectar (29.41%), and pudding was the safest one (5.88%) ($p < 0.0001$). Regarding the efficacy impairments, pudding viscosity presented the highest rate of pharyngeal residue (76.47%) when compared to nectar (58.82%) and liquid (25%) ($p = 0.01$). Indications for VFS were personalized according to the evolution and clinical setting, and mean PAS score was 4.00 ± 2.58.

3.2. Basal SSF in HV and PSD Patients

The mean SSF in all HV was 0.8 ± 0.5 swallows/min. Analyzing the results according to the age of the participants, SSF in the GI was 1.03 ± 0.62 swallows/min and was significantly reduced in GII (0.73 ± 0.50 swallows/min; $p = 0.0385$) and GIII (0.50 ± 0.31 swallows/min; $p < 0.0001$) (Figure 2A). In addition, there was a moderate significant negative correlation between age and SSF (r = −0.3810, $p < 0.0001$) (Figure 2B).

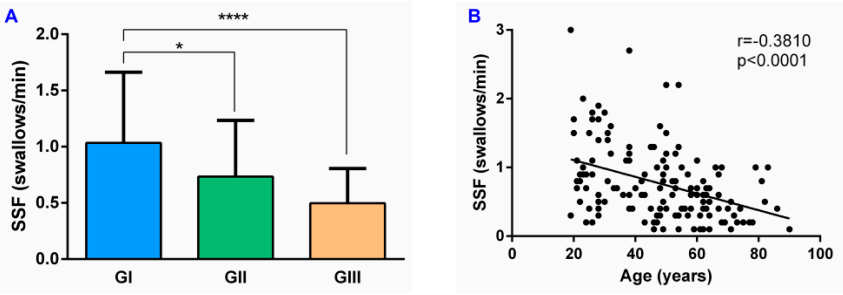

Figure 2. SSF and its correlation with age in HV: (**A**) SSF results by age groups; (**B**) correlation between SSF and age. SSF: spontaneous swallowing frequency; GI: 18–39 years age group; GII: 40–59 years age group; GIII: ≥60 years age group; * $p < 0.05$; **** $p < 0.0001$.

Regarding the effect of gender, the mean SSF in women was 0.82 ± 0.55 swallows/min and in men 0.72 ± 0.53 swallows/min ($p = 0.1846$). When comparing these results between age groups, in women there were significant differences between GI (1.01 ± 0.57 swallows/min) and GIII (0.52 ± 0.36 swallows/min; $p = 0.0067$) groups but not with GII (0.86 ± 0.56 swallows/min) (Figure 3A). In men, however, there was a significant reduction in GII (0.61 ± 0.38 swallows/min; $p = 0.0345$) and GIII (0.48 ± 0.25 swallows/min; $p = 0.0029$) when compared to GI (1.06 ± 0.67 swallows/min) (Figure 3C). A negative correlation between SSF and age was also found in women (r = −0.3534, $p = 0.0023$) (Figure 3B) and men (r = −0.3858, $p = 0.0011$) (Figure 3D). However, according to the multiple linear regression test, our data show that SSF is only affected by age ($F(1, 138) = 24.55$; $p < 0.0001$) but not by gender ($F(1, 138) = 1.347$; $p = 0.2479$).

Finally, PSD patients showed an SSF of 0.41 ± 0.32 swallows/min without significant differences when compared to the same age group in HV (GIII) ($p = 0.3112$).

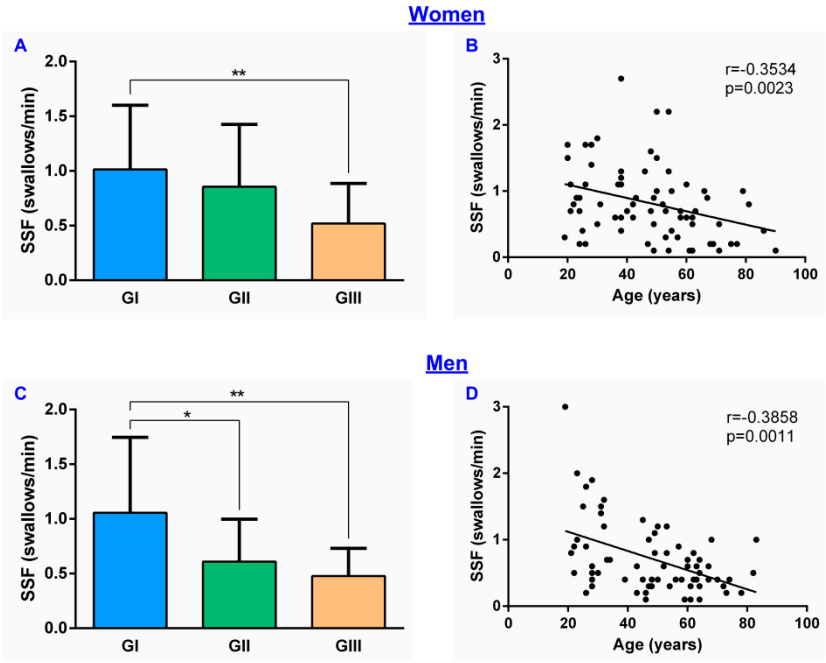

Figure 3. Gender effect on SSF: (**A**) SSF results by age groups in women; (**B**) correlation between SSF and age in women; (**C**) SSF results by age groups in men; (**D**) correlation between SSF and age in men. SSF: spontaneous swallowing frequency; GI: 18–39 years age group; GII: 40–59 years age group; GIII: ≥60 years age group; * $p < 0.05$; ** $p < 0.01$.

3.3. Basal EMG Metrics

In HV, the mean amplitude was 0.70 ± 0.61 volts, the mean delta T, 1.82 ± 0.39 s, and the mean AUC, 0.26 ± 0.37 volts·seconds. Taking into account the three age groups, we found a significant reduction only in the duration of the EMG signal (delta T) that was reduced by age between GI (1.88 ± 0.20 s) and GIII (1.74 ± 0.14 s; $p = 0.0178$) (Figure 4A). Regarding the effect of gender, significant differences between men and women were observed. Women showed a reduction in the amplitude (GII: 0.44 ± 0.11 volts, $p = 0.0158$; GIII: 0.46 ± 0.13 volts, $p = 0.0500$; vs. GI: 0.55 ± 0.11 volts) with age (Figure 4B) while in men, a reduction in delta T (GI: 2.04 ± 0.19; GII: 1.81 ± 0.15 s, $p = 0.0071$ (vs. GI); GIII: 1.66 ± 0.05, $p < 0.0001$ (vs. GI) and $p = 0.0439$ (vs. GII)) was observed (Figure 4C). No significant differences were observed for AUC.

We also found that PSD patients presented an increased amplitude (0.89 ± 0.55 volts, $p = 0.0029$) (Figure 5A), delta T (2.38 ± 0.78 s, $p = 0.0004$) (Figure 5B), and AUC (0.50 ± 0.43 volts-seconds, $p < 0.0001$) (Figure 5C) when compared to HV in GIII group with similar age.

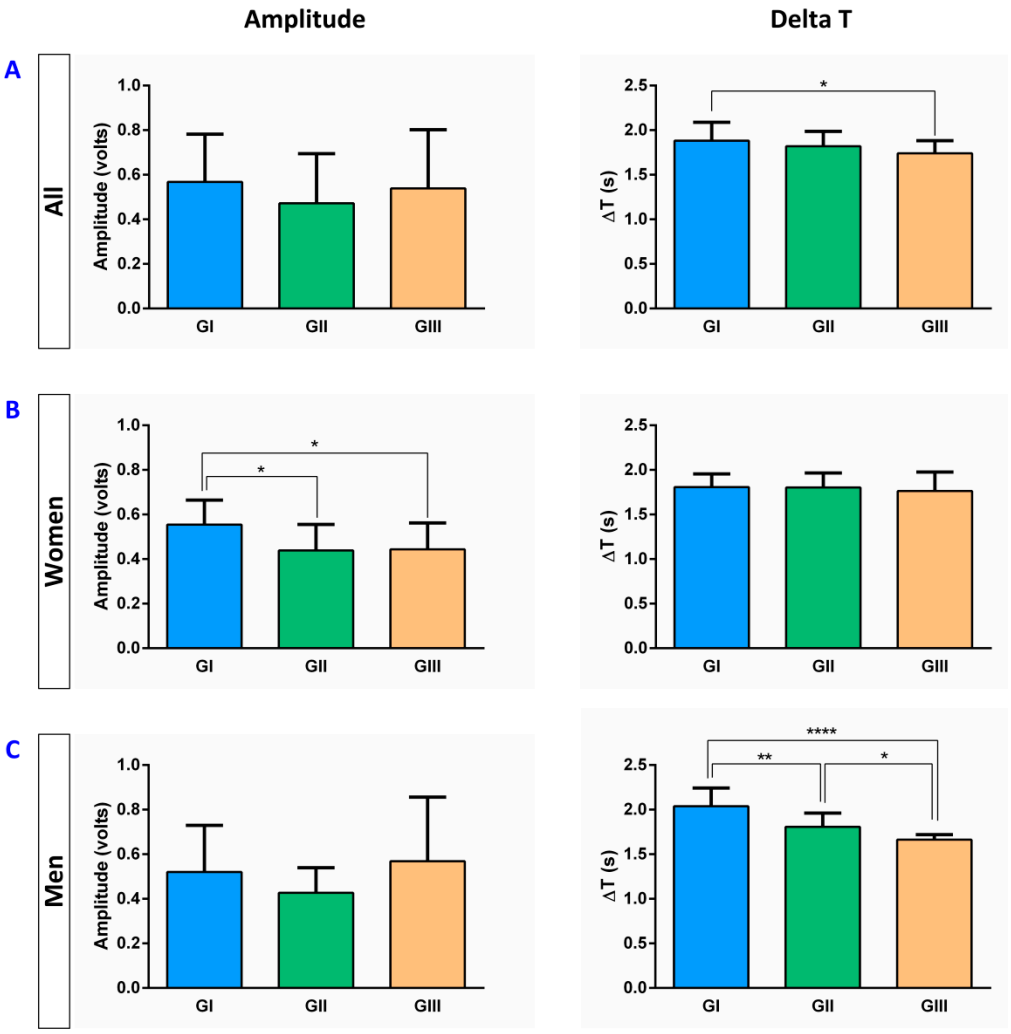

Figure 4. EMG metrics (Amplitude and Delta T) in all HV (**A**), women (**B**), and men (**C**). ΔT: Delta T; s: seconds; GI: 18–39 years group; GII: 40–59 years group; GIII: ≥60 years group; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.

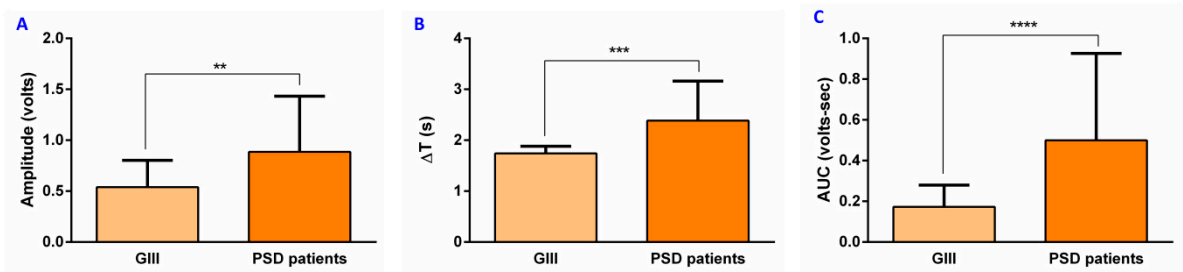

Figure 5. EMG metrics in PSD patients and GIII group: (**A**) amplitude, (**B**) Delta T, and (**C**) AUC. ΔT: Delta T; AUC: area under the curve; s: seconds; GIII: ≥60 years group; PSD: post-stroke dysphagia; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3.4. Effect of TRPV1 Stimulation on SSF and EMG Metrics in PSD

After TRPV1 stimulation with capsaicin (10^{-5} M), PSD patients showed a significant increase in SSF (0.81 ± 0.51 swallows/min, $p = 0.0003$) compared to the frequency registered before treatment (0.41 ± 0.32 swallows/min) (Figures 6A and 7). However, no significant changes in the other EMG metrics were observed: amplitude pre-treatment, 0.89 ± 0.55 volts, vs. post-treatment, 1.24 ± 0.98 volts (Figure 6B), $p = 0.1503$; delta T pre-treatment, 2.38 ± 0.78 s, vs. post-treatment, 2.30 ± 0.66 s, $p = 0.7831$; AUC pre-treatment, 0.50 ± 0.43 volts·second, vs. post-treatment, 0.59 ± 0.57 volts·second, $p = 0.8400$.

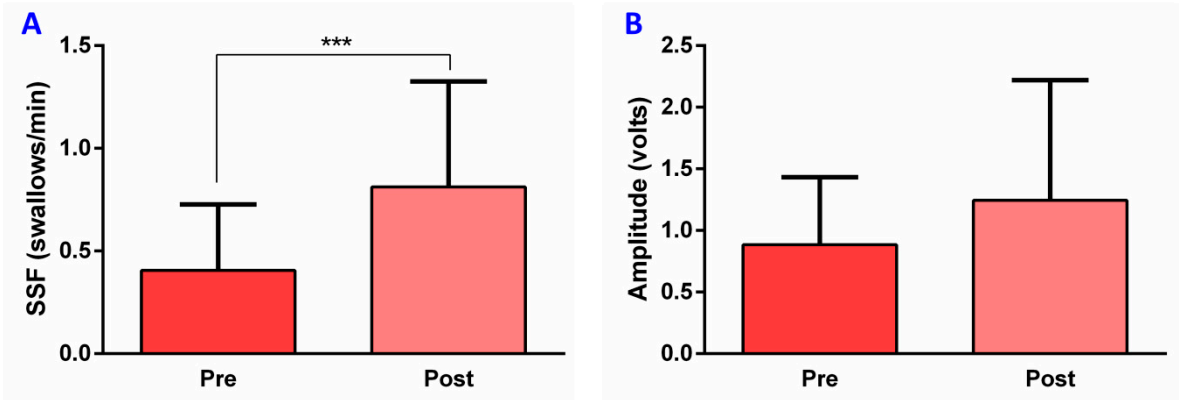

Figure 6. Capsaicin effect on SSF and EMG metrics in post-stroke dysphagia patients: (**A**) SFF, (**B**) Amplitude. SSF: spontaneous swallowing frequency; s: seconds; pre: pre-treatment; post: post-treatment; *** $p < 0.001$.

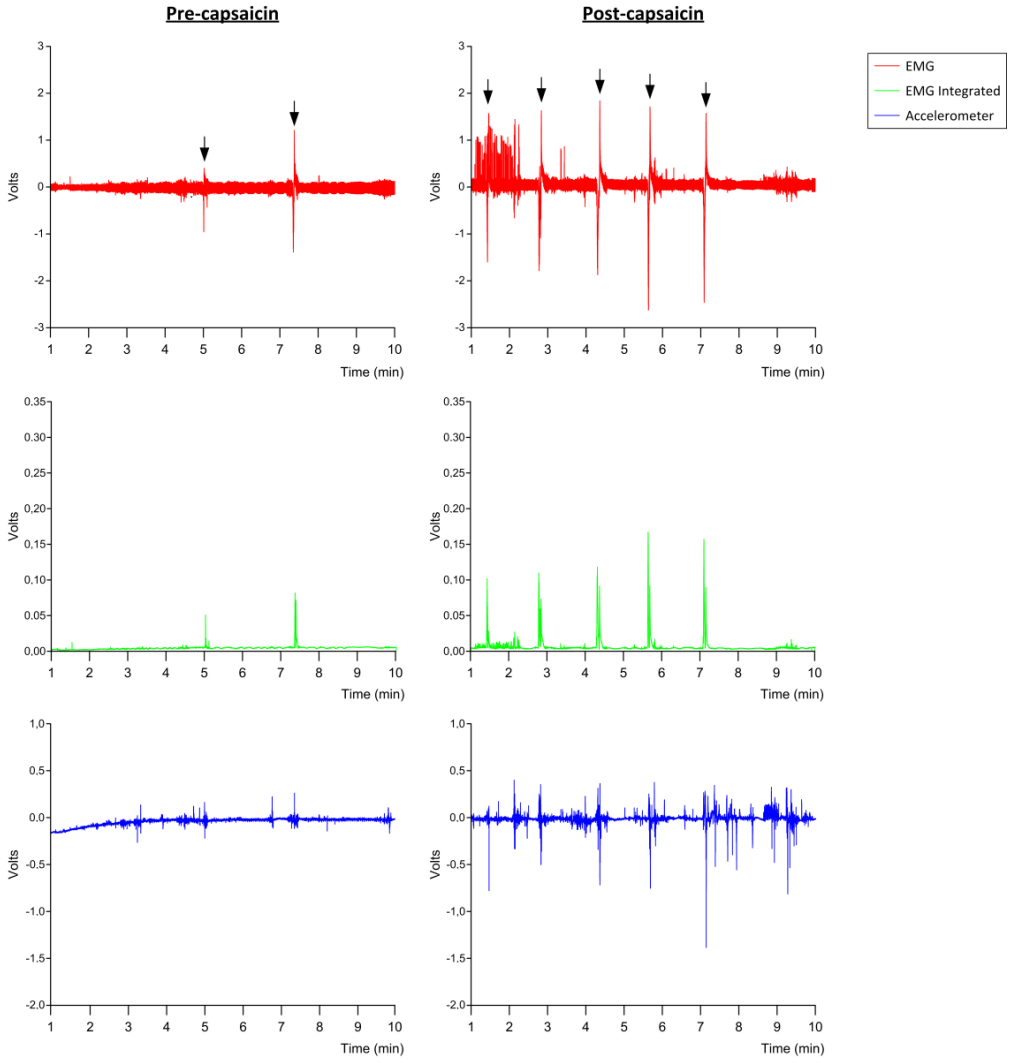

Figure 7. Pre- and post-capsaicin stimulation SSF registration. Red line represents the electromyography (EMG) signal; green line represents the EMG integrated signal; blue line represents the accelerometer movement; black arrows point to each swallow registered.

4. Discussion

The aims of this proof-of-concept study were to describe the effect of age and gender on SSF and its associated metrics, to assess whether patients with post-stroke OD had

impaired SSF and to assess the potential therapeutic effect of oropharyngeal sensory stimulation with a TRPV1 agonist such as capsaicin on SSF. In addition, we also explored the behavior of some metrics associated with SSF (amplitude, duration, AUC) in both experimental situations. We found SFF was significantly reduced by age but not by gender in HV. We also observed a trend towards reduced amplitude and duration of SSF with age. Interestingly, post-stroke dysphagic patients showed increased basal amplitude, duration, and AUC when compared to older healthy people. Acute stimulation with capsaicin caused a significant double-fold increase in SSF, further suggesting the potential role of sensory stimulation as a therapeutic strategy for CPG activation in dysphagic patients without effect on amplitude or duration.

The first result of the study was a significant reduction of SSF with age, a 29.13% reduction in GII group, and 51.47% in GIII group in comparison to GI group. We also observed that there was a significant negative correlation between age and SSF. These results concur with those previously described [9,10]. In addition, there was a reduction of the duration of each swallow in the GIII group compared to GI. Spontaneous swallowing is rhythmic motor behavior, such as breathing and sucking [26], and its motor control depends on both the peripheral and central nervous systems. Although there is no evidence of how aging impairs spontaneous swallowing, in previous studies we found that older people show a decrease in pharyngeal sensitivity, and significant alteration of the pharyngeal sensory evoked potentials, much more pronounced in older patients with OD [17]. This impairment in the sensory function is related to a reduction in the small myelinated fibers of the superior laryngeal nerve [27] and peripheral neurodegeneration of the oropharyngeal mechanoreceptors fibers, alterations that correlate with impaired mastication and swallowing function in an aging animal model [28]. We previously found that this impairment in the afferent pathway of the neurophysiological swallowing response was associated with the impaired biomechanics of oropharyngeal swallow response (OSR). Several studies from our group have shown that older people, especially those with OD, present an altered OSR, especially delayed time to LVC, which leads to an increase in the prevalence of penetrations and aspirations [29]. In this study, we found a similar effect of age on the reduction of SSF paralleling the decrease in pharyngeal sensory function and the delay in time to LVC. When we analyzed the data by age and gender, we observed that women showed a significant reduction only in SSF in GIII in comparison with the GI group. On the other hand, men showed a significant reduction in both GII and GIII groups when compared to the GI group. When we performed a multiple linear regression analysis, however, we found that SSF was only affected by the variable age but not by gender.

Regarding the EMG metrics, the amplitude, delta T, and AUC of each swallow were evaluated. The amplitude represents the maximal force contraction; the delta T, the duration of the muscular contraction; and the AUC, the integral of the amplitude and the delta T of the suprahyoid muscle contraction, showing the overall muscular effort during each swallow. We observed a reduction in the amplitude in women with age, while in men it was the duration of each swallow that was affected by age. These results agree with what has been previously described [30–32] and could be explained by the involutive changes in these muscles that are observed with aging as a consequence of different causes such as malnutrition or sarcopenia [33]. Taken together, we hypothesize this reduction in SSF with age in healthy volunteers parallels the decrease in the pharyngeal sensory function of these persons.

Regarding the SSF in patients with post-stroke OD, no significant differences were found when compared to the GIII HV group (HV of the same age). In contrast to what was previously described [11,12], we did not observe significant differences on SSF between HV and post-stroke patients with OD due to the reduced number of patients included in the present study and also to the combination of acute and chronic patients. In contrast, we observed significant changes in the EMG metric, with a significant increase in the amplitude, the duration, and the AUC of the muscular contraction associated with each swallow. The increased duration of each swallow could be related to the prolonged neurophysiological

and biomechanical response (time from GPJO-LVO) of swallowing reported in post-stroke patients [34]. In addition to a delayed oropharyngeal and prolonged swallow response, these patients showed a loss of symmetry of the pharyngeal sensory evoked potentials and their cortical representation [18], and a reduced and delayed pharyngeal motor evoked potentials [35]. About the increase in amplitude, few studies have related the increase in this variable in older patients with OD to the loss of adipose tissue, specifically in the submental region, as fat attenuates the EMG signals [32,36]. Another interpretation could be that these patients with post-stroke OD require a stronger muscular effort to elicit a spontaneous swallow.

The hypothesis that the neural control of spontaneous swallowing depends on the CPG and could be similar to that described for the blink reflex [2,3,7,8], leads to peripheral neurostimulation strategies as a possible treatment to improve SSF. One of the most used chemical strategies to improve swallow response is capsaicin stimulation. This natural agonist activates TRPV1 receptors, which are widely located in the epithelial cells and nerve endings of the human oropharynx and could be activated by endogenous or exogenous agonists [15]. After acute treatment with capsaicin 10^{-5} M, post-stroke patients showed a significant increase in SSF without any major changes in the EMG metrics, probably due to the small sample. In a previous study in healthy subjects, significant changes in pharyngeal and UES function were assessed by manometric and EMG metrics after an acute treatment with capsaicinoids at the same concentration used in our study [37]. Our group also has demonstrated the therapeutic effect of capsaicin at different concentrations and acute/subacute administration. Regarding the acute (single dose) treatment, we observed an improvement in time to LVC and UESO, reduction in the prevalence of oral and pharyngeal residue, and increased cortical excitability at a concentration of 150 μM but not at 10^{-5} M [19,22,38]. The low dose showed better results when patients received the treatment over 10 days, three times a day. In this case, patients not only showed improvements in biomechanics but also in neurophysiology, inducing a faster and more intense response by shortening the latency, and increasing the amplitude of the pharyngeal sensory evoked potential peaks. In addition, we described a significant correlation between the improvement of N1 peak latency and the improvement of time to LVC, suggesting that neuroplasticity processes were being induced that resulted in improved OSR [21,22]. With this evidence, we can conclude that capsaicin would be inducing greater conduction of stimuli through the afferent pathway to the CPG, resulting in an enhancement of the SSF.

This proof-of-concept study had some limitations. First of all, the sample size of patients with post-stroke OD is quite small, combined patients in the acute and chronic state, and swallowing function was only assessed with V-VST. We are working on another prospective study where a larger sample of patients with an acute stroke and OD will be included. In addition, a group of acute post-stroke patients without OD will also be included. Further studies are needed to explore the SSF in OD patients to know if it could be used as a screening tool. Finally, it would be interesting to study if there are changes in the concentration of salivary SP after capsaicin treatment and if it is related to the improvement observed in SSF. Additionally, the duration of the improvement in SSF following TRPV1 stimulation with capsaicin should be measured.

5. Conclusions

Spontaneous swallowing frequency ranged around one swallow per minute in healthy young volunteers, which was significantly affected by age but not by gender. Post-stroke patients with OD showed no differences in SSF compared with HV of similar age but presented higher amplitude and longer duration of each spontaneous swallowing. Acute stimulation with capsaicin results in a significant increase in SSF, suggesting the involvement of oropharyngeal TRPV1 receptors on the swallowing reflex control and further supporting a potential pharmacological strategy to treat post-stroke patients with OD with these pharmacological compounds.

Supplementary Materials: The following are available online at https://www.mdpi.com/2075-4418/11/3/461/s1: Figure S1. Placement of the electrodes (blue arrow) and accelerometer (red arrow). Figure S2. Example of a swallowing signal. The EMG signal is shown in red, the EMG integrated in green and the accelerometer in blue. EMG: electromyography.

Author Contributions: P.C. and W.N. designed the research study. W.N., S.A., C.C. and C.C.-A. performed the research. N.T. and W.N. analyzed the data. N.T., W.N., M.A.-L., and P.C. wrote the article. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Hospital de Mataró on 17th November 2017 (protocol code 11/17).

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Data Availability Statement: The data presented in this study are available within the article and their supplementary material.

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Acute and subacute effects of oropharyngeal sensory stimulation with TRPV1 agonists in older patients with oropharyngeal dysphagia: a biomechanical and neurophysiological randomized pilot study

Noemí Tomsen, Omar Ortega, Laia Rofes, Viridiana Arreola, Alberto Martin, Lluís Mundet and Pere Clavé

Abstract

Background: Older people with oropharyngeal dysphagia (OD) present a decline in pharyngeal sensory function. The aim of this proof-of-concept study was to assess the biomechanical and neurophysiological effects of acute and subacute oropharyngeal sensory stimulation with transient receptor potential vanilloid 1 (TRPV1) agonists (capsaicinoids) in older patients with OD.

Methods: We studied the effect of a single dose *versus* multiple doses (2 weeks) of oral capsaicin treatment (10^{-5} M) or placebo in 28 older patients with OD (81.2 ± 4.6 years) using videofluoroscopy (penetration-aspiration scale [PAS], timing of swallow response) and electroencephalography (EEG) (latency and amplitude of pharyngeal event-related potential [ERP]).

Results: Acute stimulation by capsaicinoids 10^{-5} M did not improve swallow function and did not produce significant changes in pharyngeal ERP. In contrast, after 10 days of treatment, patients presented a clinically relevant and statistically significant reduction in the laryngeal vestibule closure (LVC) time (22.5%, $p = 0.042$), and in the PAS (24.2%, $p = 0.038$), compared with the placebo group. EEG results showed a reduction in the latency of the N1 peak (28.6%, $p = 0.007$) and an increase of the amplitude of the P1-N2 (59.4%, $p = 0.038$) and the N2-P2 (43.6%, $p = 0.050$) peaks. We observed a strong and significant correlation between the reduction in the latency of the N1 peak and change in LVC time after subacute treatment ($r = 0.750$, $p = 0.003$).

Conclusions: After 2 weeks of treatment, oropharyngeal sensory stimulation with capsaicinoids induced cortical changes that were correlated with improvements in swallowing biomechanics in older patients with OD. These results further show that sensory stimulation by TRPV1 agonists can become a useful pharmacological treatment for older patients with OD.

Keywords: deglutition disorders, neurophysiology, oropharyngeal dysphagia, therapeutics

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Correspondence to:
Pere Clavé
Laboratori de Fisiologia Digestiva CIBERehd CSdMUAB, Hospital de Mataró, Barcelona, Spain

Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Instituto de Salud Carlos III, Barcelona, Spain

Fundació Institut d'Investigació Germans Trias i Pujol, Badalona, Spain
pere.clave@ciberehd.org

Noemí Tomsen
Omar Ortega
Laboratori de Fisiologia Digestiva CIBERehd CSdM-UAB, Hospital de Mataró, Barcelona, Spain

Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Instituto de Salud Carlos III, Barcelona, Spain

Laia Rofes
Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Instituto de Salud Carlos III, Barcelona, Spain

Viridiana Arreola
Alberto Martin
Lluís Mundet
Laboratori de Fisiologia Digestiva CIBERehd CSdM-UAB, Hospital de Mataró, Barcelona, Spain

Introduction

Oropharyngeal dysphagia (OD) is a prevalent disorder among older people that leads to severe complications and impaired quality of life.[1,2] OD has recently been recognized as a geriatric syndrome by two European societies as it is highly prevalent in the older population, caused by multiple risk factors, associated with several comorbidities and poor prognosis, and needs a multidisciplinary approach to treatment.[3] The pathophysiology of

OD in the older population has been studied and the impairment in the oropharyngeal swallow response (OSR) is well known.[4,5] Instrumental diagnostic methods such as videofluoroscopy (VFS) have enabled the quantitative measurement of the timing of the OSR[4,6] to better understand its pathophysiology.[7–9] The OSR in older patients is weak and slow, leading to impaired efficacy and safety of swallow. Impaired efficacy (mainly oropharyngeal residue) is caused by weak tongue bolus propulsion, diminished tongue base movements, reduced pharyngeal muscular reserve, lower width opening of the upper oesophageal sphincter (UOS) and slow hyoid motion caused by muscular weakness and sarcopenia: impaired safety (penetrations and aspirations) is mainly due to delayed laryngeal vestibule closure (LVC) and slow neural response.[4,10–12] The motor pathway of the neural control of swallow has been characterized using transcranial magnetic stimulation, clarifying the cortical control of swallowing muscles.[13,14] However, the role of the afferent (sensory) pathway of deglutition is less well known although some studies suggest it has a key role in the pathophysiology of OD.[15–17] Recently we explored the pharyngeal event-related potential (ERP) to electrical stimulation in several groups (young healthy volunteers and older patients with and without OD) and found older people had a decline in pharyngeal sensory function, that was more severe in older patients with OD.[17] This sensory impairment might be a critical pathophysiological element and a potential target for treating swallowing dysfunction in older patients. This hypothesis was confirmed in one of our studies where we found that administration of acute transient receptor potential vanilloid 1 (TRPV1) agonists (capsaicinoids (1.5^{-4} M)) improved swallow function (reduced penetrations and penetration-aspiration scale [PAS]) and the biomechanics of the swallow response (LVC, UOS opening and hyoid movement) in a group of older patients with OD.[18] However this high concentration is pungent and not suitable for long-term studies. For this reason, we performed a subacute study (10 days treatment) with capsaicinoids at a lower dose (10^{-5} M) and found an improvement in the safety of swallow and OSR in up to 68.42% of older patients with OD without any complaints from the patients regarding pungency.[5] Regarding the subacute and acute treatments, it is important to state that the effect remained stable over the treatment period and they did not lose their effectivity due to desensitization. Some other studies have found that sensory stimulants, such as TRPV1 agonists, improved swallowing parameters.[19–23] Despite these promising results it is not known if and how capsaicin improves the neurophysiology of the afferent pathway, which is crucial in order to design future therapies for older patients with OD.

We hypothesized that subacute administration of pharyngeal sensory stimulants, such as capsaicinoids, would improve cortical neuroplasticity, measured by pharyngeal ERP to electrical stimulation (latency/amplitude). This improvement would lead to a faster and greater conduction and integration of the sensory input resulting in a faster and stronger swallow response. The aim of this proof-of-concept study was to assess the effect of acute (single-dose protocol) and subacute (multiple-dose protocol) treatment with capsaicinoids on the biomechanics and neurophysiology of older patients with OD and to assess any possibility of desensitization after 2 weeks of treatment.

Methods

Study population

A total of 28 older patients with OD associated with aging were included in the study and patients in each study were randomized with a specific software (QuickCalcs 2018, GraphPad Software) (Supplementary Figure 1). Inclusion criteria were for patients to be more than 70 years old, in a stable medical condition and to have clinical signs of OD according to the volume-viscosity swallow test.[24] The study protocol was approved by the Ethics Committee of the Hospital de Mataró (protocol code CEIC04/12) and was conducted according to the principles and rules laid down in the Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from all the participants. ClinicalTrials.gov registration code: NCT01762228.

Study design

Studies were conducted in the Dysphagia Unit of the Hospital de Mataró (Barcelona, Spain). Two study protocols were designed to evaluate the effects of acute and subacute treatment with oral capsaicinoids 10^{-5} M on the neurophysiology of older patients with OD (Supplementary Figure 2).

Acute study, single-dose protocol. A total of 14 patients with OD were included in this protocol

and randomized to capsaicinoids or placebo intervention. First, socio-demographic and clinical data were collected: age, sex, Barthel index[25] (functional status), Mini Nutritional Assessment short form[26] (nutritional status) and Charlson index[27] (comorbidities). All participants were examined with VFS to assess their swallowing function and, after 5 days, underwent an electroencephalographic (EEG) study to explore the ERP while they received the TRPV1 agonist or placebo[17] (Supplementary Figure 2).

Subacute study, multiple-dose protocol. A total of 14 patients with OD (> 70 years) were included in this protocol and randomly allocated to the TRPV1 agonist or placebo group. All participants were studied with VFS and EEG before and 5 days after the treatment (Supplementary Figure 2).

Intervention: oropharyngeal sensory stimulation with TRPV1 agonists. In the single-dose protocol, participants were given 10 ml of a nectar-like solution of capsaicinoids 10^{-5} M (active group), as previously published,[5] or placebo (potassium sorbate, the excipient of the capsaicinoids solution) (control group). In the multiple-dose protocol, participants were given 10 ml of a nectar-like solution of capsaicinoids 10^{-5} M or placebo three times/day (before meals) for 2 weeks (5 days/week). Capsaicinoid concentration in the capsaicinoids sauce (McIlhenny Co, Avery Island, LA, USA) was 185.5 μg/g, measured with liquid chromatography (AOAC 995.03 method).[18]

VFS/swallow physiology

VFS procedure. All patients were imaged seated, in a lateral projection which included the oral cavity, pharynx, larynx and cervical oesophagus. VFS recordings were obtained with a SuperXT-20 Toshiba Intensifier (Toshiba Medical Systems Europe, Zoetermeer, the Netherlands) using a continuous fluoroscopy beam and recorded at 25 frames/s using a Panasonic AGDVX-100B video camera (Matsushita Electric Industrial Co, Osaka, Japan).

Acute studies. The single-dose protocol has been described previously.[18] Patients were studied during the deglutition of one series of 5 ml, 10 ml and 20 ml nectar-viscosity boluses (274.42 ± 13.14 mPa·s) as a control and two series of 5 ml, 10 ml and 20 ml nectar boluses supplemented with capsaicinoids (10^{-5} M). A sensitization process on each patient was conducted 5 min before treatment by giving two 5 ml boluses supplemented with capsaicinoids (10^{-5} M) 2 min apart (Supplementary Figure 2).

Subacute studies. To assess the effect of subacute administrations of capsaicinoids on the biomechanics of swallow, we performed two VFS (pre- and post-treatment). Patients were studied during the deglutition of 5 ml, 10 ml and 20 ml series of nectar (274.42 ± 13.14 mPa·s), liquid (20.40 ± 0.23 mPa·s) and pudding boluses (3931.23 ± 166.15 mPa·s). Liquid viscosity was obtained by mixing 1:1 mineral water and the X-ray contrast Gastrografin (Bayer Hispania SL, Barcelona, Spain). Nectar and pudding viscosity were obtained by adding 3.5 g and 8 g, respectively, of the thickener Resource ThickenUp (Nestlé Nutrition, Barcelona, Spain) to 100 ml of liquid (1:1 water/contrast). Boluses were carefully given to patients with a syringe.[5]

VFS analysis. VFS analysis was carried out blind by a single observer. A good inter-rater correlation has been described in the assessment of the signs of impaired safety of swallow we used in this study ($\kappa = 0.7051$) and intra-rater (0.9) and inter-rater (0.9) reliability for the timing of OSR.[28–30] Digitization, analysis and measurements of VFS images were made using the software Swallowing Observer (Image and Physiology SL, Barcelona, Spain). For each swallow, we analysed: (a) signs of swallowing efficacy; the presence of oral or pharyngeal (vallecular and pyriform sinus) residue was assessed; (b) signs of swallowing safety: laryngeal vestibule penetrations and tracheobronchial aspirations, classified according to PAS, were assessed.[4,31] Quantitative measurements of the OSR were obtained during 5 ml swallows: timing of the LVC and UOS opening were measured, the glossopalatal junction opening was given the time value 0[4] (Supplementary Figure 3).

EEG/ERP

Electroencephalographic procedure.

Single-dose protocol: the EEG consisted of two trains of 50 squared wave 0.2 ms electrical stimuli applied to the pharynx with an intrapharyngeal catheter passed transnasally, with two bipolar electrodes (Gaeltec Ltd, Dunvegan, Scotland) 1 cm apart. The electrodes were positioned to the

posterior pharyngeal wall, 14–15 cm from the nostrils and the catheter was connected to a Digitimer DS7A current stimulator and DG2A train/delay generator (Digitimer Ltd, Welwyn Garden City, UK). The stimulus intensity was individualized for each participant by increasing the electrical current intensity from 0 mA in steps of 0.5 mA, first determining the threshold intensity at which the participants perceived the stimulus (sensory threshold) and then increasing to the tolerance threshold. Thresholds were determined by triplicate and the intensity applied to assess the ERP was 75% of the tolerance threshold. The inter-stimulus interval was 5 s and the inter-set interval, 1 min. Following the two trains, participants were randomized into two groups. One was given a single dose of 10 ml capsaicinoids 10^{-5} M while the other received 10 ml placebo. Then, two new trains of stimuli with the same characteristics were applied to the pharynx of all participants (Supplementary Figure 2).

Multiple-dose protocol: EEG protocol was performed as previously described after the subacute 10 days of treatment. During each EEG, participants did not receive any stimulant between the four trains of stimuli (Supplementary Figure 2).

Pharyngeal ERP recording. Cortical responses to electrical stimuli were recorded through a cap with 32 scalp tin electrodes (Electro-Cap International Inc, Eaton, OH, USA), an amplification of the 10–20 system,[32] referenced to the left ear lobe and connected to a BrainAmp amplifier (Brain Products GmbH, Gilching, Germany). The ground electrode was included in the EEG cap and located just below the fronto-central electrode FCz. A disc electrode was placed on the skin below the left eye to record the vertical electrooculogram. Electrode gel was applied to the electrodes to keep impedance below 5 KΩ. The signal was digitized at a sampling rate of 500 Hz and filtered with a 50 Hz notch. Recordings were performed in a quiet room with the subject seated, awake and with eyes open. The subject was asked to stay calm and relaxed.

Pharyngeal ERP analysis. The EEG was analysed offline and processed with BrainVision Analyzer Software 2.0 (Brain Products GmbH) in the following steps: the EEG was bandpass filtered between 0.5 Hz and 60 Hz; an independent component analysis was performed to correct eye blink artefacts; the EEG was segmented into 600 ms epochs, including 100 ms of prestimulus baseline; a semi-automatic artefact-rejection method was employed to prevent contamination from swallow movements; the interval from –100 ms to –20 ms before the stimulus was used for baseline correction; the epochs were averaged to obtain the pharyngeal ERPs for each participant, and the responses of the participants of each group were averaged. Cortical representation of the ERPs was shown using the registries from the software. The following time frames were used to compute cortical activity distribution for the different peaks: N1: 56–80 ms; P1: 120–150 ms; N2: 220–270 ms; P2: 300–350 ms.[17] We have shown the N1 and P2 peaks in the figures as they are the first (afferent conduction) and last of the peaks (cortical integration), respectively.

ERP source localization. To identify the topography of the brain source of each ERP component, the standardized low-resolution brain electromagnetic tomography software (sLORETA) (http://www.uzh.ch/keyinst/loreta.htm) was used to compute a standardized, discrete, three-dimensionally distributed, linear, minimum norm inverse solution. The particular form of standardization used provides the exact localization to test point sources, giving images of standardized current density but low spatial resolution.[17]

Safety of the treatment

During the study, no adverse events or serious adverse events were recorded and reported to the Ethics Committee of the Hospital de Mataró.

Statistics

Continuous variables are expressed as mean ± SD and categorical variables as relative and absolute frequencies. Continuous variables were compared by the Student's t test (for inter-group comparisons) and by the paired t test (for pre/post-test comparisons) or Friedman test (for control/T1 and T2 treatment time points comparisons). Categorical variables were compared by Fisher's exact test. Nonparametric tests were used when appropriate (non-Gaussian variables). Correlation between LVC time and the latency of N1 pharyngeal ERP was determined with Spearman's correlation coefficient. p value < 0.05 was considered statistically significant. After 10 days of treatment, patients were divided into responders and nonresponders. Responders were defined as those

Table 1. Socio-demographic and clinical characteristics of the population in the acute and subacute arms of the study. Data presented as mean ± SD unless specifically stated.

	Acute study			Subacute study		
	Group 1: placebo	**Group 2: capsaicinoids**	***p* value**	**Group 1: placebo**	**Group 2: capsaicinoids**	***p* value**
N	7	7	1.000	7	7	1.000
Age (years)	83.7 ± 3.9	83.5 ± 6.3	0.530	79.0 ± 5.7	79.8 ± 5.2	0.680
Sex (*n*, men)	5	4	1.000	5	4	0.550
Barthel index	79.2 ± 25.4	70 ± 33.7	0.567	55 ± 39.1	85.8 ± 16.8	0.200
MNA-sf	12.3 ± 1.9	9.5 ± 2.9	0.106	8.3 ± 3.8	11.7 ± 2.9	0.200
Charlson index	3.7 ± 2.6	3.8 ± 2.5	0.959	2.8 ± 1.9	2.0 ± 1.3	0.570

MNA-sf, Mini Nutritional Assessment short form.

patients who, following treatment, achieved safe swallow at a lower level of viscosity or improved at least one point in the PAS at the same viscosity.[5] The sLORETA software package was used to assess the differences in cortical localization between pre/post-treatment results computed by voxel-by-voxel *t* tests for paired measures and corrected for multiple comparisons.[17]

Results

Acute stimulation: single-dose protocol

Study population. A total of 14 older patients with OD (82.9 ± 3.2 years, 9 men) were included in this arm of the study. Patients randomized to active (Group 2) or placebo (Group 1) treatment had similar ages, number of comorbidities, functional capacity and nutritional status (Table 1).

VFS results. VFS results showed a population with a high prevalence of VFS signs of impaired efficacy of swallow (mainly pharyngeal residue) and low prevalence of VFS signs of impaired safety of swallow (penetrations) with a moderately delayed timing of OSR. After the single-dose treatment with capsaicinoids, patients did not show any significant improvement when compared with the pretreatment values (Table 2).

EEG results. The sensory threshold to electrical stimulation was 13.0 ± 5.4 mA for Group 1 and 11.3 ± 4.5 mA for Group 2 ($p = 0.410$). The level of intensity at which the stimulus was applied was similar between groups (20.6 ± 7.8 mA for Group 1 and 22.9 ± 12.0 mA for Group 2, $p = 0.870$). A single dose of capsaicinoids 10^{-5} M did not produce significant changes in the pharyngeal ERP (amplitude and latency) (Supplementary Table 1, Figure 1). We found no correlations between the biomechanics and neurophysiology of swallow response in the acute study.

ERP source localization. In the acute study, no statistical differences were found. In patients who received placebo, the N1 peak distribution showed a weak activation of the occipital lobe, the P1 peak showed a bilateral frontal distribution, the N2 peak had a bilateral frontotemporal cortical representation, and P2 had a right frontal activation (Figure 1). In the capsaicin group, after treatment, there was a centralization of the cortical activation in the N1 peak, P1 and N1 peaks had an increased cortical representation in the left frontotemporal area and finally, P2 had a moderate increase in the activation of the right frontal lobe.

Using sLORETA, we found the basal anatomical activation in both groups of treatment, showing different localizations according to each one of the peaks from the pharyngeal ERPs: N1 peak activation was found in the middle temporal gyrus (Brodmann area [BA] 21); P1 and P2 in the medial frontal gyrus (BA10); N2 in the inferior frontal gyrus (BA47).

Table 2. Swallowing characteristics of the seven patients that received the active single-dose treatment. Data presented as % except for PAS score, LVC and UOS opening time (mean ± SD).

	Pretreatment ($n = 7$)	Post-treatment		p value
		T1	T2	
Impaired efficacy (%)	85.7	57.1	57.1	0.424
Oral residue (%)	42.9	42.9	42.9	1.000
Pharyngeal residue (%)	85.7	42.9	42.9	0.174
Impaired safety (%)	28.6	28.6	0.0	0.291
Penetrations (%)	28.6	28.6	0.0	0.291
Aspirations (%)	0.0	0.0	0.0	1.000
PAS score	1.6±1.1	2±1.4	1±0.0	0.296
LVC time (ms)	251.4±72.0	245.7±106.9	211.43±44.5	0.302
UOS opening time (ms)	228.6±64.1	228.6±72.0	211.4±59.8	0.539

LVC, laryngeal vestibule closure; PAS, penetration-aspiration scale; UOS, upper oesophageal sphincter.

Compared with basal activation, patients that received the active treatment showed a significant reduction in cortical activity at the N1, P1 and N2 peaks ($p = 0.0002$) distributed in the following way: the N1 peak showed less activation at the anterior cingulate (BA24); P1 and N2 peaks at the paracentral lobule (BA6, premotor cortex); P2 at the cuneus (BA17, primary visual cortex).

Subacute stimulation: multiple-dose protocol

Study population. A total of 14 additional older patients with OD were included in this arm of the study (79.4 ± 5.2 years, 8 men), with similar socio-demographic and clinical characteristics between patients that received the placebo (Group 1) and patients that receive capsaicinoids (Group 2) (Table 1).

VFS results

Safety signs. All patients showed impaired safety of swallow (PAS > 2) before receiving any treatment. After 10 days of treatment with capsaicinoids, patients presented a significant reduction in the PAS from 4.14 ± 0.4 to 3.14 ± 0.9 ($p = 0.038$) without changes in the prevalence of aspirations and penetrations. Patients in Group 1 (control group) did not show changes (Table 3). According to the established definition we found a responder rate of 71.43% (5) in Group 2 (capsaicinoids group) and of 28.57% in Group 1 (2), but there were no significant differences in responder rate between capsaicinoids and control groups ($p = 0.2861$).

Efficacy signs. We observed alterations in the efficacy of 100% of the participants. Neither the patients treated with capsaicinoids nor the control group presented changes after treatment (Table 3).

Oropharyngeal swallow response. Patients in the control group (Group 1) did not present changes in the timing of the OSR. In contrast, patients treated with capsaicinoids (Group 2), showed a statistically significant reduction in the LVC time from 457.3 ± 46.8 ms to 354.3 ± 53.8 ms ($p = 0.042$) and the UOS opening time from 348.6 ± 88.6 ms to 285.7 ± 78.66 ms ($p = 0.125$) (Table 3).

EEG results. The sensory threshold of patients included in Groups 1 and 2 was 11.0 ± 3.1 mA and 7.0 ± 5.4 mA, respectively ($p = 0.173$). The intensity level at which the stimulus was applied did not change between sessions: Group 1 received a stimulation intensity of 24.5 ± 8.1 mA before treatment and 24.2 ± 8.5 mA after treatment ($p = 1.00$) and Group 2 received a

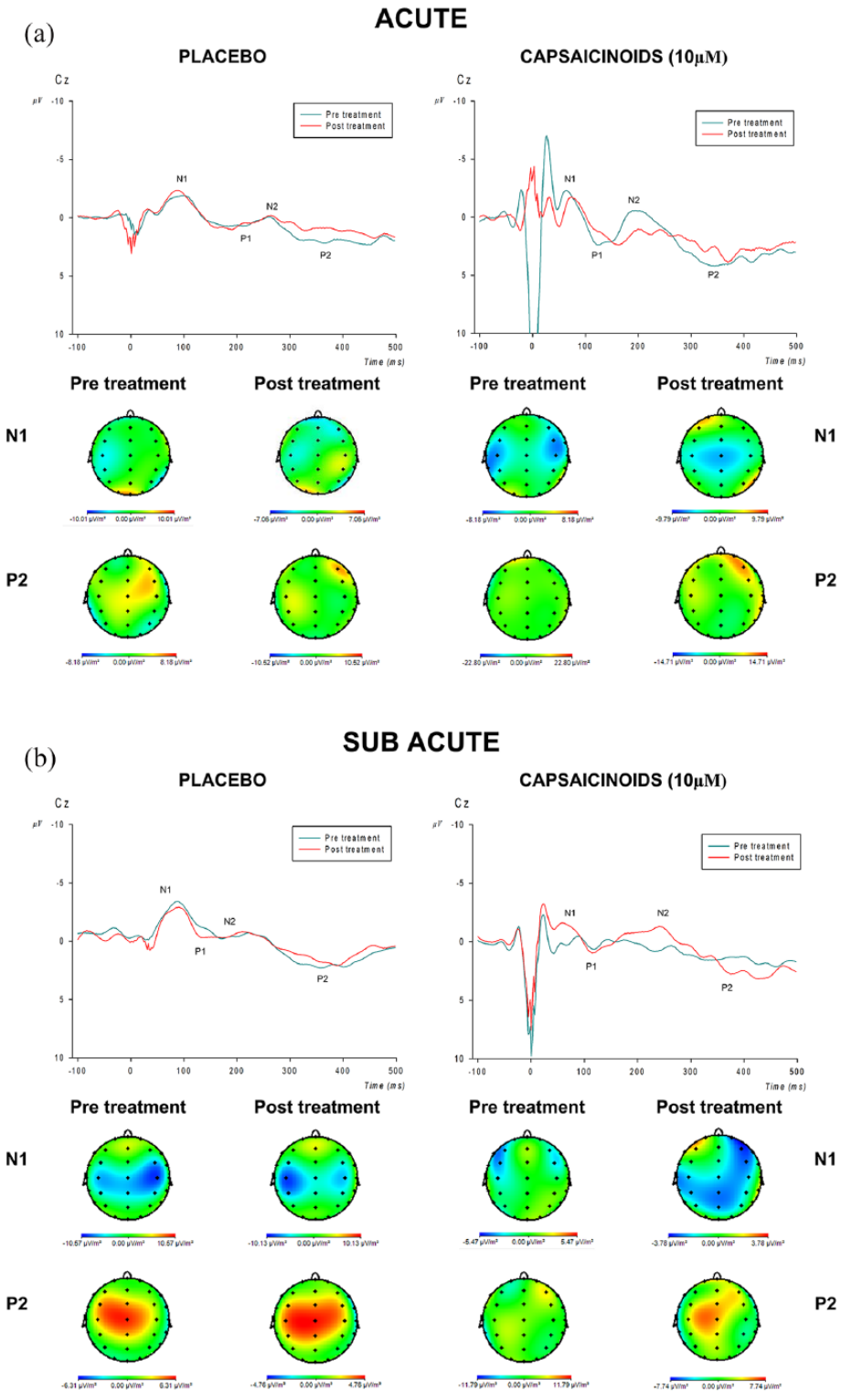

Figure 1. Pharyngeal event-related potential (ERP) and scalp density maps to pharyngeal ERPs for acute (a) and subacute (b) studies. At the top of each treatment, ERP traces obtained at the Cz electrode for pretreatment (blue line) and post-treatment (red line) from the placebo group and capsaicinoids group after pharyngeal electrical stimulation are shown. Deflection at time point 0 corresponds to stimulus artefact. At the bottom, current scalp density maps at each ERP peak time point for each group are shown.

Table 3. Swallowing characteristics and latency and peak-to-peak amplitude of the pharyngeal ERP components at the Cz electrode of the placebo and capsaicinoids groups.

	Group 1: placebo		*p* value	Group 2: capsaicinoids		*p* value
	Pretreatment $n = 7$	Post-treatment $n = 7$		Pretreatment $n = 7$	Post- treatment $n = 7$	
Swallowing characteristics (VFS)						
Impaired efficacy (%)	85.7	85.7	1.000	100	85.7	1.000
Oral residue (%)	85.7	85.7	1.000	85.7	71.4	1.000
Pharyngeal residue (%)	71.4	57.1	1.000	85.7	71.4	1.000
Impaired safety (%)	100	100	1.000	100	71.4	0.462
Penetrations (%)	71.4	100	0.462	100	71.4	0.462
Aspirations (%)	28.6	14.3	1.000	0	0	1.000
PAS Score	4.9 ± 1.2	5 ± 1.0	1.000	4.14 ± 0.4	3.14 ± 0.9	0.038
LVC time (ms)	342.9 ± 142.1	388.6 ± 88.6	0.469	457.1 ± 46.8	354.3 ± 53.8	0.042
UOS opening time (ms)	274.3 ± 90.7	274.3 ± 81.4	0.936	348.6 ± 88.6	285.7 ± 74.6	0.125
Pharyngeal ERP (EEG)						
N_1 latency (ms)	85.7 ± 15.4	81.7 ± 16.9	0.463	90 ± 18.6	64.3 ± 16.8	0.007
P_1 latency (ms)	153.7 ± 35.0	157.4 ± 31.8	0.403	128 ± 20.6	112.3 ± 24.5	0.325
N_2 latency (ms)	234.9 ± 37.4	218.9 ± 38.5	0.219	241.7 ± 68.9	257.4 ± 44.9	0.295
P_2 latency (ms)	341.7 ± 35.3	333.4 ± 58.7	0.437	374 ± 70.9	388.6 ± 66.0	0.623
P_1-N_1 amplitude (μV)	3.6 ± 1.3	3.5 ± 1.3	0.850	2.2 ± 1.7	3.2 ± 1.7	0.311
P_1-N_2 amplitude (μV)	0.5 ± 1.1	1.1 ± 2.5	0.504	1.3 ± 1.4	3.2 ± 1.9	0.038
N_2 -P_2 amplitude (μV)	3.2 ± 4.0	3.4 ± 3.6	0.784	3.1 ± 1.0	5.5 ± 2.3	0.050

EEG, electroencephalography; ERP, event-related potential: LVC, laryngeal vestibule closure; PAS, penetration-aspiration scale; UOS, upper oesophageal sphincter; VFS, videofluoroscopy.

stimulation of 16.08 ± 5.1 mA before treatment and 15.7 ± 5.6 mA after treatment ($p = 0.872$).

A 2-week treatment with capsaicinoids induced significant changes in the pharyngeal ERP; a reduction in the latency of the N1 peak (from 90.0 ± 18.6 ms to 64.3 ± 16.8 ms, $p = 0.007$) and an increase in the amplitude of the P1-N2 (from 1.3 ± 1.4 mV to 3.2 ± 1.9 mV, $p = 0.038$) and N2-P2 (from 3.1 ± 1.0 mV to 5.5 ± 2.3 mV, $p = 0.050$) peaks (Figure 1, Table 3). In contrast, patients in Group 1 did not show any change in their pharyngeal ERP or their cortical activation after the 2-week treatment (Figure 1, Table 3). Pretreatment and post-treatment scalp distributions of the pharyngeal ERP showed no statistically significant differences in Group 1 (Table 3).

Correlation between VFS and EEG results. The reduction observed in the latency of the N1 component of the pharyngeal ERP after the subacute treatment strongly and significantly correlated ($r = 0.750$; $p = 0.003$) with the reduction observed in the LVC time, the main airway protection mechanism during swallow. In contrast there was no correlation when we analysed the reduction observed

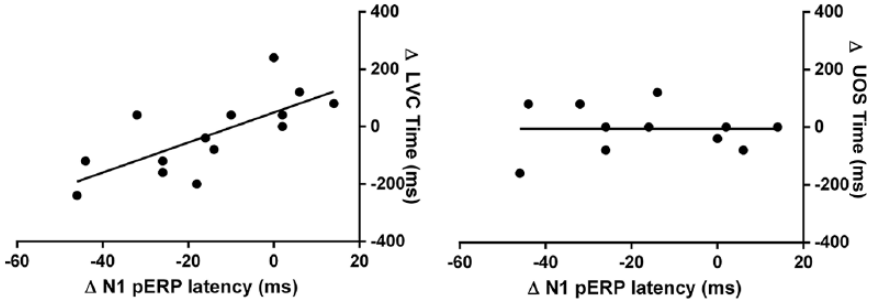

Figure 2. Correlation between the change in LVC time (left) and UOS closure time (right) and the reduction in the latency of N1 peak after the multiple-dose treatment.
LVC, laryngeal vestibule closure; pERP, pharyngeal event-related potential; UOS: upper oesophageal sphincter.

in the latency of the same peak with that of the overall duration of the swallow response until the UOS closure time ($r = -0.06026$; $p = 0.7503$) (Figure 2).

ERP source localization. Patients that received the placebo in the subacute study did not show changes in cortical activation distribution. The N1 peak had a bilateral cortical representation; the P1 peak had a frontoparietal and left frontal lobe representation; the N2 peak had a right temporal and frontoparietal distribution; the P2 peak had a wide centroparietal representation (Figure 1). After 2 weeks of treatment with capsaicinoids, we also found statistically significant differences (Table 3). N1 cortical activation changed from bilateral frontal distribution to a frontoparietal and temporal distribution; P1 cortical representation (not shown) changed from a unilateral frontal representation to a more bilateral frontal distribution; N2 temporal bilateral activity was reduced; P2 activity was strongly increased with higher representation in the frontoparietal lobes (Figure 1).

The basal anatomical activation was similar to that described in the acute study. The localization of each peak was: BA21 in the N1 peak, BA10 in the P1 and P2 peak, and BA47 in the N2 peak.

After 2 weeks of treatment with capsaicinoids, we observed an increase in cortical activation at the N1, N2 and P2 peaks ($p < 0.0001$) represented as increased activity in the N1 and P2 peaks on the cingulate gyrus (BA31), in P1 at the paracentral lobule (BA5, somatosensory association cortex), and N2 at the medial frontal gyrus (BA8 secondary motor cortex) (Figure 3).

Safety of the treatment

During the study there were no adverse or serious adverse events. Thus, we concluded that our treatments were safe for our older patients with OD.

Discussion

Results from this study further confirm that the biomechanical airway protection mechanisms during the swallow response (mainly LVC) are delayed in older patients with OD and the cortical activation to pharyngeal sensory stimuli at ERP is also delayed, impaired and reduced in this population. We also found that acute treatment with low doses of capsaicinoids (10 μM) did not have any effect on the OSR or the pharyngeal ERP. In contrast, subacute treatment with the same concentration of capsaicinoids induced significant cortical changes that correlated with significant improvements in the OSR of older patients with OD, further suggesting that the use of sensory stimulation by TRPV1 agonists could be a valid pharmacological strategy for these patients. However, although our results concur with our initial hypothesis, we need to perform further studies and a randomized clinical trial with more patients to confirm that these neuroplastic and biomechanical changes in swallow function are caused specifically by subacute TRPV1 stimulation.

Our research strategy first characterized the impaired biomechanics of the OSR in older people using VFS and then the therapeutic effect of oropharyngeal sensory stimulation using TRP agonists. We found that impaired safety of deglutition and aspirations in older people are mainly caused by delayed LVC.[4] We also found that acute oropharyngeal sensory stimulation with natural capsaicinoids (150 μM) had the strongest therapeutic

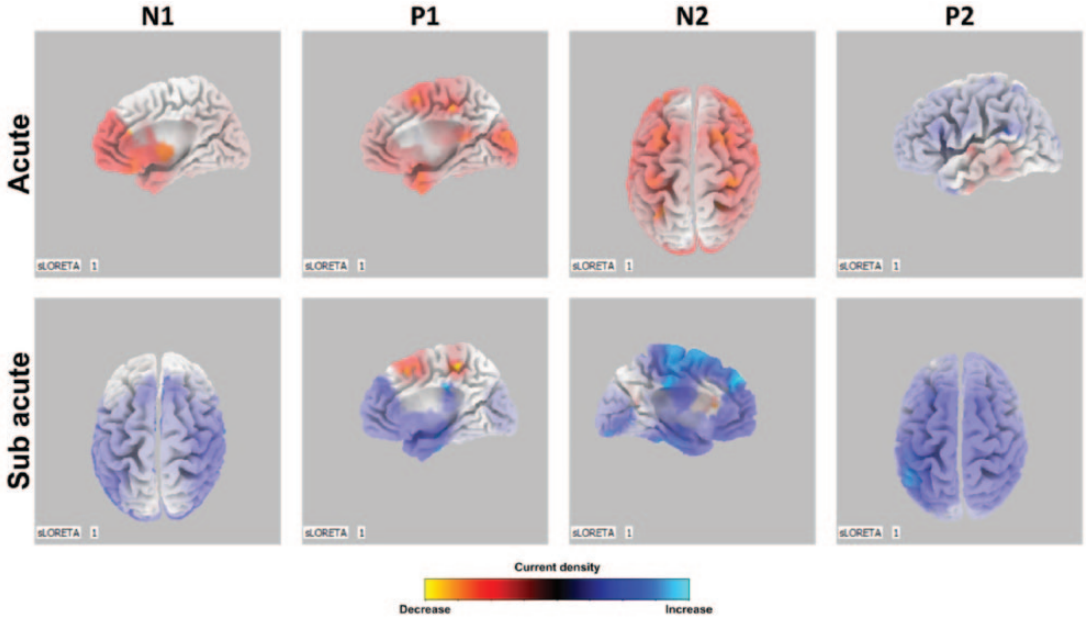

Figure 3. Differences in LORETA source activity after acute stimulation (top) and subacute stimulation (bottom) compared with basal cortical activity. Coloured voxels represent areas of significant differences in activation (blue, increase; red, decrease) after correction for multiple comparisons.

effect over other TRP agonists by significantly reducing the prevalence of penetrations, pharyngeal residue, LVC time and increasing bolus velocity in older patients with OD.[18,23] However, in the present study we did not find such an improvement in VFS results when applying acute stimulation with capsaicinoids at lower doses. This lower concentration (10 μM) compared with the previous study (150 μM)[18] was due to the poor palatability felt by patients at this concentration due to pungency, and by the good results found in a previous subacute study with a 10 μM dose in older patients with OD with a positive responder rate of 68.42%.[5] Moreover, the application of the same reduced concentration of capsaicinoids for 10 days significantly reduced the severity of impaired safety alteration measured by the PAS in 24.15% ($p = 0.038$) and the most relevant biomechanical element of the reconfiguration phase of the OSR, the LVC ($p = 0.042$) (~100 ms reduction). In addition we found a responder rate of 71.43%, similar to that of our previous subacute study,[5] further suggesting the strong therapeutic effect of this sensory-stimulation approach to developing future pharmacological treatments for OD.

We previously found that the impaired conduction/integration of the afferent pathway in cerebral structures involved in sensory pharyngeal processing was closely associated with the pathophysiology of OD in older patients.[17] The preservation of the afferent pathway is essential for a safe and effective swallow, allowing continuous oropharyngeal feedback to higher-level cerebral centres and activation of sensorimotor integration processes.[33] In previous studies with a similar methodology, we found that older patients with OD had an impaired cortical response to the pharyngeal electrical stimulus compared with older people without dysphagia. The amplitude of all peaks was clearly inferior in older patients with OD, while only the latency of the N1 and N2 peaks was delayed,[17] with values quite similar to those observed in the present study. We did not find any significant effect of acute stimulation on the latency or amplitude of ERP. In contrast, when we analysed pharyngeal ERP in the subacute study, we also found a significant reduction in the latency of the N1 peak, and an increase in the amplitude of the P1-N2 and N2-P2 and changes in cortical activation after capsaicinoids treatment, indicating an improvement in conduction (N1 and P1 peaks) and integration (N2 and P2 peaks) of sensory information into the cortex. This suggests that capsaicinoids treatment induces plastic cortical changes that are translated into a safer and faster OSR.

As we reported in a previous study, older patients with OD show an increase in prefrontal and associated-area activation to compensate for the impaired activation of the other brain areas involved in the OSR.[17] When we analysed brain activity with sLORETA, we found a similar increase in frontal activation prior to the stimulation treatment. After acute stimulation, we found a reduction in brain activity in nonrelevant areas for deglutition. However, after subacute capsaicinoids stimulation, we observed more activity in the cingulate gyrus (limbic lobe) at the N1 and P2 peaks and in the medial frontal gyrus (frontal lobe) at the N2 peak. Both areas, especially the cingulate gyrus, are mainly activated during swallowing preparation and play a major role in the perception of stimulus and go/no-go decisions,[34] suggesting a neuroplasticity potential for future OD treatments based on sensory stimulation.

We also found a strongly significant correlation between LVC reduction time and N1 pharyngeal ERP peak latency reduction, indicating that biomechanical improvements seen with VFS correlate with neurophysiological improvements on the sensory side. On the other hand, we did not find this correlation when we compared the total duration of swallow with the same peak latency. This result agrees with a previous publication of our group, which found that, after the application of acute capsaicinoids treatment, the LVC time was significantly reduced but the treatment did not modify the total duration of OSR.[18] This indicates that this stimulant affects the first phase of pharyngeal swallow (airway protection mechanisms and reconfiguration from a digestive to a respiratory pathway).

Other studies have also found a close relationship between sensory deficits and impaired OSR, indicating that reduced sensory input is translated into an impaired motor response in patients with OD.[16,35,36] This pathophysiologically relevant factor in OD has been shown to be reversed or improved with our treatment, suggesting the relevance of sensory stimulation in the management and treatment of older patients with OD and indicating that subacute administration of capsaicinoids is a valid strategy to induce cortical changes that have a positive impact on swallow physiology. Despite these good results, we still need to adjust the dosage to obtain the highest biomechanical improvements with the minimal-dose effect due to the pungency of capsaicinoids. It is also important to note that there were no desensitization effects after 2 weeks of treatment.

Finally, there are some studies that correlate the increase in substance P (SP) level in saliva after pharyngeal sensory stimulation.[37] A recent publication showed that after oral capsaicin treatment (0.7 μg during 7 days), there was an increase in salivary concentration of SP that was associated with an improvement in the safety and efficacy of swallowing, and with a shortening in the OSR in older patients with OD.[19] These results suggest that SP can be used as a biomarker for neurostimulation treatment response to stimulants such as capsaicin and will be an interesting factor to take into account in future studies.

This study has some limitations, the main one being that this is a proof of concept with few patients in each group. Further studies with larger sample sizes will be needed in order to confirm these results and with a second post-treatment neurophysiological evaluation at a longer follow-up time period to assess whether the observed neuroplasticity is maintained over time. In addition, patients from the acute study had a lower severity of OD compared with those in the subacute study and this could have affected the results of the study as those patients from the acute study had less potential improvement margin than those from the subacute. To solve this limitation, in future studies with a similar design, we will randomize patients to the acute or subacute group instead of randomizing only for treatment or placebo to balance the severity of OD between both therapeutic groups.

Conclusion

We have shown that impaired pharyngeal sensory function is a key element in the pathophysiology of dysphagia in older patients and treatments increasing the sensory input, such as TRPV1 agonists, will play a major role in the future treatment of dysphagia. The acute effect of capsaicin and capsaicinoids has been well studied by our group but the chronic effect is not well known and this study shows an improvement in this direction.

Future studies with larger patient samples, and including salivary SP measurement, are needed to better learn the chronic effects of these stimulants and to select the most appropriate dose to improve dysphagia and avoid nutritional and respiratory

complications among older patients with OD. The therapeutic paradigm for older patients with OD is now changing from compensatory to improving brain and swallow function.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

Supplementary material

Supplementary material for this article is available online.

ORCID iD

Noemí Tomsen https://orcid.org/0000-0001-7149-7948

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ORIGINAL ARTICLE

Oropharyngeal Dysphagia in Older People is Associated with Reduced Pharyngeal Sensitivity and Low Substance P and CGRP Concentration in Saliva

Noemí Tomsen[1,2,3] · Omar Ortega[1,2] · Weslania Nascimento[2] · Silvia Carrión[2] · Pere Clavé[1,2]

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Abstract

Substance P (SP) and Calcitonine gene-related peptide (CGRP) are released by sensory nerve fibers in the oropharynx. Patients with oropharyngeal dysphagia (OD) present reduced oropharyngeal sensitivity and low SP concentration in saliva. We aimed to assess the concentration of salivary SP and CGRP in healthy volunteers, and older people without and with OD, and the relationship with pharyngeal sensory threshold. We included 15 healthy volunteers, 14 healthy elderly and 14 elderly with OD. Swallow function was assessed by videofluoroscopy (VFS). Pharyngeal sensory threshold was assessed by intrapharyngeal electrical stimulation. Hydration and phase angle were assessed by bioimpedance. Saliva samples were collected with a Salivette® to determine SP and CGRP concentration by ELISA. Elderly patients with OD presented impaired safety of swallow (PAS 4.38 ± 0.77 $p < 0.0001$ vs. healthy volunteers = 1 and healthy elderly = 1.43 ± 0.51). Healthy elderly and elderly with OD presented a reduction in intracellular water and saliva volume (healthy elderly, 592.86 ± 327.79 µl, $p = 0.0004$; elderly with OD, 422.00 ± 343.01 µl, $p = 0.0001$ vs healthy volunteers, 1333.33 ± 615.91 µl, $r = 0.6621$, $p < 0.0001$). Elderly patients with OD presented an impairment in pharyngeal sensory threshold (10.80 ± 3.92 mA vs. healthy volunteers, 5.74 ± 2.57 mA; $p = 0.007$) and a reduction in salivary SP (129.34 pg/ml vs. healthy volunteers: 173.89 pg/ml; $p = 0.2346$) and CGRP levels (24.17 pg/ml vs. healthy volunteers: 508.18 pg/ml; $p = 0.0058$). There was a negative correlation between both SP and CGRP concentrations and pharyngeal sensory threshold ($r = -0.450$, $p = 0.024$; $r = -0.4597$, $p = 0.036$, respectively), but only SP identified elderly patients with OD with higher pharyngeal sensory threshold. Elderly patients with OD presented hydropenia and sarcopenia, reduced salivary SP and CGRP and impaired pharyngeal sensitivity. Our study suggests SP levels in saliva as a potential biomarker to monitor pharyngeal sensitivity in elderly patients with OD.

Keywords Deglutition disorders · Aged · Neuropeptides · Substance P · Afferent pathways · Pharyngeal sensitivity

Introduction

Oropharyngeal dysphagia (OD) is a prevalent condition among older people [1]. OD is included in the International Classification of Diseases of the WHO [2] and is recognized as a geriatric syndrome by relevant European scientific societies [3]. Impaired safety of deglutition and aspirations in older people are mainly caused by delayed laryngeal vestibule (LV) closure whereas impaired efficacy and residue are mainly related to weak tongue bolus propulsion forces [4]. In addition, older people present a decline in pharyngeal sensory function, more severe in those with OD, that also impair the pharyngo-cortical connection and disrupt patterns of sensory cortical activation [5].

The impairment in pharyngeal sensory function observed during aging is associated with peripheral neurodegeneration

✉ Pere Clavé
pere.clave@ciberehd.org

[1] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas Y Digestivas (Ciberehd), Instituto de Salud Carlos III, Barcelona, Spain

[2] Gastrointestinal Motility Laboratory, Department of Surgery, Hospital de Mataró, Consorci Sanitari del Maresme, Universitat Autònoma de Barcelona, Carretera de Cirera s/n 08304, Mataró, Spain

[3] Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

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of the oropharyngeal mechanoreceptors [6] and a reduction in small myelinated nerve fibers of the superior laryngeal nerve (SLN) [7]. In animal studies, these alterations correlate with impaired mastication and swallowing function [6]. The oropharynx is a highly innervated area with specialized structures and receptors involved in sensory perception. The main receptors are the Transient Receptor Potential (TRP) channels: the subfamily vanilloid 1 (TRPV1), the subfamily ankyrin 1 (TRPA1) and the subfamily melastatin 8 (TRPM8), located in the membrane of the epithelial cells and the sensory nerves (C-fibres and Aδ fibres) that innervate the mucosa and submucosa [8, 9]. All these receptors are sensitized by mechanical, thermal and chemical stimuli found in the alimentary bolus. TRP activation initiates the sensory input transmission through cranial nerves V, VII, IX and X and the release of some neuropeptides including Substance P (SP) and Calcitonin gene-related peptide (CGRP) from these sensory terminals [10, 11].

Similar mechanisms involving SP and CGRP release can be found in other areas of human physiology. Most of the corneal nerves are sensory and originate from the ophthalmic branch of the V nerve. SP is present in human tears, and age-associated corneal nerve loss is paralleled by a reduction in SP expression. SP has trophic effects in the long term and modulates nociception in the cornea and, in animal models, reduced corneal sensitivity was restored by topical SP treatment [12]. CGRP has also been located in the sensory nerves in the animal and human cornea, suggesting a similar physiological role [13, 14]. Studies on animals and humans suggest similar modulation pathways for cough and swallow. Stimulation of two subtypes of primary sensory neurons can induce coughing: (a) C-fiber nociceptors, with terminals in and around the mucosa surface of the airways, that are sensitive to inhaled or locally produced chemical mediators including TRP agonists, and (b) mechanically sensitive "cough receptors" positioned beneath the epithelium in the large airways and are particularly sensitive to mechanical stimuli delivered to the mucosa (for example, inhaled particulate matter). In this model, the inhaled sensory stimuli activates the TRP receptors located in C-fibers, which release SP and CGRP that stimulate the mechanoreceptor fibres, facilitating the transmission of the sensory inputs to the solitary tract nucleus and the sensory cortex for the cough reflex [15, 16].

Previous studies in our laboratory have shown that concentration of SP is reduced in the saliva of older patients with OD compared with patients without OD [17, 18]. In addition, the concentration of SP, along with other neuropeptides, is increased in saliva after several acute peripheral neurostimulation treatments for OD, such as intrapharyngeal electrical stimulation [19] and oral capsaicin [20, 21], suggesting that SP could serve as a biomarker for sensory neurostimulation treatment response. In contrast, there is no information on the release of CGRP in saliva in dysphagic patients. Thus, these salivary neuropeptides could become a complementary diagnostic element in selecting patients with impaired oropharyngeal sensitivity who are potential "responders" to specific sensory neurorehabilitation treatments for OD [22, 23]. Our previous studies showed that addition of TRPV1, TRPV1/A1 or TRPM8 agonists to the bolus improved the swallow response [22, 24–26] in association with a significant improvement in sensory cortical activation [23, 27]. We also described a subset of "patient responders" to these new sensory neurorehabilitation strategies [22, 23].

The aim of this study was to describe a non-invasive biomarker for patients with impaired oropharyngeal sensory function, hypothesizing that there was a correlation between the salivary SP and CGRP with pharyngeal sensitivity We measured the salivary concentration of SP and CGRP and their relation to pharyngeal sensory and motor function in young healthy volunteers and older people with and without OD to determine whether it would serve as a biomarker to identify patients with impaired oropharyngeal sensitivity who are potential candidates for sensory neurorehabilitation strategies.

Methods

Study Population

A total of 43 participants were included in the study: 15 young (18–55 years) healthy volunteers, 14 older (> 65 years) people without OD and 14 older people with OD. Inclusion criteria were to be aged between 18 and 50 years for the healthy volunteers and more than 65 years for the healthy elderly and elderly with OD, in a stable medical condition, without history of neurologic or neurodegenerative diseases for the healthy elderly and to have impaired safety of swallow (PAS > 2) according to videofluoroscopy (VFS) for the elderly with OD. The study was approved by the Ethical Committee of the Hospital de Mataró (code 3/17) and was conducted according to the rules of the Declaration of Helsinki and its last amendments. All the participants included in the study signed the informed consent.

Study Design

The study was conducted in the Gastrointestinal Motility Laboratory of the Hospital de Mataró and consisted of only one visit. Healthy elderly and elderly with OD were screened with the V-VST to determine clinical signs of OD and assessed by VFS. Those with a Penetration-Aspiration Scale (PAS) [28] score higher than 2 during VFS were classified as elderly with OD. Socio-demographic and clinical

data (functional status: Barthel index [29]; nutritional status: Mini Nutritional Assessment short form (MNA-sf) [30]; and comorbidities: Charlson index [31]) and a sample of saliva were collected from all participants. A bioimpedance study was also performed to assess body composition and hydration status of study individuals. Finally, the pharyngeal sensory threshold to intrapharyngeal electrical stimulation was determined in each participant by triplicate.

Videofluoroscopy Procedures

A VFS was performed on all participants. The dynamic images of swallow were obtained while the study subjects were seated in lateral projection and included the oral cavity, the pharynx, the larynx and the cervical oesophagus as previously described [4]. Participants were studied during the deglutition of 5, 10 and 20 ml of liquid, nectar (274.42 ± 13.14 mPa/s) and pudding (3931.23 ± 166.15 mPa/s) viscosity boluses. All boluses were obtained mixing 1:1 water and Gastrografin (Bayer Hispania SL, Barcelona, Spain), adding 3.5 g or 8 g of Resource ThickenUp (Nestlé Nutrition, Barcelona, Spain) to obtain nectar and pudding respectively. Several parameters were analyzed during the 5 ml nectar swallow: (a) the presence of oral and pharyngeal (pyriform sinus and vallecula) residues; (b) the occurrence and severity of penetrations and aspirations, classified according to the PAS score; (c) and the kinematics of the swallow response by measuring the timing from GPJO time to laryngeal vestibule closure (LVC) and to upper esophageal sphincter opening (UESO) [4].

Assessment of Body Composition and Hydration Status

Body composition was measured with the Bioimpedance Analyzer Modul BIA 101 (Akern SRL, Pontassieve, Italy) and results obtained with the BiaVector® software (Akern SRL, Pontassieve, Italy). Body composition parameters included the percentage of total water, extracellular water, intracellular water, cellular mass, fat mass, lean mass, muscle mass, the phase angle (in degrees) and the basal metabolism (Kcal).

Saliva Collection and Measurement of SP and CGRP

Saliva samples were gathered at the same time of day (from 8 am to 12 pm) from all participants to avoid circadian rhythm effect. The collection was made using a Salivette® (Sarstedt, Nuembrecht, Germany) by placing the swab under the tongue of the participant for 5 min and then centrifuging it immediately at 2600 rpm for 2 min. The supernatants were stored at -80ºC until their analysis. SP and CGRP concentration was assessed using two specific commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) kits: Substance P Parameter Assay Kit (ref. KGE007; R&D systems, Minneapolis, MN, USA) and CGRP (Human) ELISA kit (ref. KA5439; Abnova, Taipei City, Taiwan).

Pharyngeal Sensory Threshold

The pharyngeal sensory threshold to electrical stimulation was determined for each participant. The electrical stimuli were applied through an intrapharyngeal catheter with two bipolar electrodes (Gaeltec Ltd, Dunvegan, Scotland) that were positioned at 14–15 cm from the nostril [5]. The catheter was connected to Digitimer DS7A current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom). The sensory threshold was determined by triplicate, applying several stimuli of 0.2 ms at increasing intensity in steps of 0.5 mA until patients' first perception of the electrical stimulus.

Data Analysis and Statistical Methods

The minimum detectable SP and CGRP concentration in saliva samples of the ELISA kits was used (161 pg/ml and 25 pg/ml, respectively) and we divided the patients in groups above or below these concentrations of salivary SP or CGRP [32]. The upper limit of pharyngeal sensory threshold reference interval was calculated from the mean pharyngeal sensory threshold of the healthy volunteers plus two standard deviations. All statistical tests were performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Continuous variables were compared with a one-way ANOVA test and expressed as mean ± standard deviation (SD). A multiple comparison post test was performed to describe any differences between the three groups. Categorical data were compared with the chi-square test and expressed as relative and absolute frequencies. Nonparametric tests were used when appropriate. The correlations were determined with Spearman's correlation coefficient. Significant differences were considered when p value < 0.05.

Results

Study Population

The study included 43 participants divided into three groups with a similar distribution of men and women. Most patients in the healthy elderly and elderly with OD groups presented a preserved functional status, were well nourished and had a low number of comorbidities, without significant differences between groups (Table 1).

Table 1 Socio-demographic and clinical data of the HV, HE and EOD groups. Data expressed as mean ± SD

	HV	HE	EOD
n	15	14	14
Sex (men:women)	7:8	5:9	7:7
Age (years ± SD)	32.73 ± 8.34	74.36 ± 6.49****	79.14 ± 6.37****
Barthel index	100	95.00 ± 8.99	97.86 ± 6.71
MNA-sf	14	13.14 ± 1.35	12.57 ± 1.79
Charlson index	0	1.50 ± 1.22	1.07 ± 0.62

HV healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia, *MNA-sf* Mini Nutritional Assessment short form

****$p < 0.0001$ compared with HV

VFS Assessment of Swallow Function

All the study participants were assessed with VFS. Healthy volunteers showed no signs of impaired safety and/or efficacy, with a mean time to LVC and to UESO of 188.00 ± 45.23 ms and 157.33 ± 43.99 ms respectively. Up to 78.57% of healthy elderly had mild efficacy impairments (oral and/or pharyngeal residue), with a mean PAS score of 1.43 ± 0.51 and with preserved mean time to LVC and to UESO (221.82 ± 60.30 ms and 218.18 ± 45.13 ms, respectively) [4]. In contrast, all patients classified as elderly with OD presented signs of both impaired efficacy and safety of swallow and a mean PAS score of 4.38 ± 0.77. In addition, elderly patients with OD presented a significant delay in the time to LVC (346.67 ± 78.78 ms, $p = 0.0004$) and to UESO (276.67 ± 77.14 ms, $p = 0.024$) when compared to healthy elderly (Table 2).

Body Composition, Hydration Status and Saliva Volume

Bioimpedance measurements showed that healthy elderly and elderly with OD presented significant changes in body composition, mainly an increased fat mass and a reduced lean mass and muscular mass vs healthy volunteers. Elderly with OD also presented a reduction in the cellular mass (Table 3). Both healthy elderly and elderly with OD showed significant hypertonic dehydration as the relationship between the extracellular water and the intracellular water was significantly increased in healthy elderly (1.36 ± 0.38, $p = 0.0014$) and elderly with OD (2.31 ± 1.35, $p = 0.0002$) and the phase angle was significantly reduced (healthy elderly: 3.79 ± 1.40°, $p = 0.0049$; elderly with OD: 2.77 ± 1.86°, $p = 0.0017$) when compared with healthy volunteers (extracellular water/intracellular water: 0.66 ± 0.22; phase angle: 6.55 ± 2.30°) as a consequence of the reduction in intracellular water (Fig. 1a). In addition, the volume of the saliva sample collected was smaller in healthy elderly and elderly with OD (healthy volunteers: 1333.33 ± 615.91 ml vs healthy elderly: 592.86 ± 327.79 ml, $p = 0.0004$; elderly with OD: 422.00 ± 343.01 ml, $p = 0.0001$) (Fig. 1b) showing a strong positive correlation between saliva volume and hydration status assessed by phase angle ($r = 0.6621$, $p < 0.0001$) (Fig. 1c).

Pharyngeal Sensory Threshold

The pharyngeal sensory threshold in healthy volunteers was 5.75 ± 2.48 mA, which was poorer in healthy elderly (7.57 ± 2.53 mA, $p = 0.6897$) and significantly impaired in elderly with OD (10.80 ± 3.72 mA, $p = 0.007$), clearly showing a reduced pharyngeal sensitivity in elderly patients with OD. Up to 14.29% of healthy elderly and up to 35.71% of

Table 2 HV, HE and EOD VFS results

	HV	HE	EOD
Efficacy impairment (%)	0	78.57****	100****
Oral residue (%)	0	57.14***	64.29***
Pharyngeal residue (%)	0	57.14***	78.57****
Safety impairment (%)	0	0	100****;####
Penetrations (%)	0	0	100****;####
Aspirations (%)	0	0	7.14
PAS score	1	1.43 ± 0.51	4.38 ± 0.77****;####
Time to LVC (ms)	188.00 ± 45.23	221.82 ± 60.30*	346.67 ± 78.78****;###
Time to UESO (ms)	157.33 ± 43.99	218.18 ± 45.13	276.67 ± 77.14***

Data expressed as mean ± SD

HV Healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia; *PAS* penetration-aspiration scale, *LVC* laryngeal vestibule closure, *UESO* upper esophageal sphincter opening

****$p < 0.0001$ compared to HV, ***$p < 0.001$ compared to HV, *$p < 0.05$ compared to HV, ####$p < 0.0001$ compared to HE, ###$p < 0.001$ compared to HE, #$p < 0.05$ compared to HE

Table 3 Bioimpedance and body composition

	HV	HE	EOD
Total water (%)	52.41 ± 7.31	51.22 ± 17.86	50.51 ± 7.24
ECW (%)	42.15 ± 13.83	56.76 ± 6.17**	65.63 ± 12.15***
ICW (%)	57.85 ± 13.83	43.24 ± 6.17**	34.22 ± 12.07***
ECW/ICW	0.66 ± 0.22	1.36 ± 0.38**	2.31 ± 1.35***
PA (°)	6.55 ± 2.30	3.79 ± 1.40**	2.77 ± 1.86**
Na/K	0.85 ± 0.27	1.09 ± 0.39	0.98 ± 0.54
Cellular mass (%)	54.58 ± 12.38	46.21 ± 17.10	33.48 ± 11.62**
Fat mass (%)	25.63 ± 7.49	41.91 ± 6.63****	36.83 ± 9.05**
Lean mass (%)	74.37 ± 7.49	58.09 ± 6.63****	63.18 ± 9.05**
Muscular mass (%)	51.69 ± 2.50	34.27 ± 9.86**	28.72 ± 8.14***
Basal metabolism (Kcal)	1599.49 ± 169.04	1295.04 ± 176.67*	1261.72 ± 174.38*

All data are expressed as mean ± SD

HV healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia, *ECW* extracellular water, *ICW* intracellular water, *PA* phase angle

****$p < 0.0001$ compared to HV, ***$p < 0.001$ compared to HV, **$p < 0.01$ compared to HV, *$p < 0.05$ compared to HV

elderly with OD had pharyngeal sensory threshold above the upper limit of the reference interval (10.71 mA).

SP and CGRP Concentrations in Saliva and Its Correlation with Pharyngeal Sensitivity

Regarding the salivary concentration of the neuropeptides, healthy elderly (168.36 ± 109.26 pg/ml) and elderly with OD (139.96 ± 47.42 pg/ml) showed a non-significative decrease in SP when compared with healthy volunteers (192.34 ± 116.57 pg/ml) (Fig. 2a), while the reduction of CGRP was significant in both older groups (healthy elderly: 45.07 ± 40.43 pg/ml, $p = 0.0089$; elderly with OD: 24.17 ± 6.99 pg/ml, $p = 0.0058$; vs healthy volunteers: 508.18 ± 286.29 pg/ml) (Fig. 2c).

There was a moderate negative correlation between the pharyngeal sensory threshold and salivary SP concentration ($r = -0.450$, $p = 0.024$) and CGRP ($r = -0.4597$, $p = 0.036$) (Fig. 2b and d). In addition, the pharyngeal sensory threshold was higher in those healthy volunteers and elderly with OD participants with the lowest salivary SP levels (healthy volunteers: 7.80 ± 1.36 mA; elderly with OD: 8.97 ± 2.35 mA) than in those with higher SP levels (healthy volunteers: 3.94 ± 1.36 mA, $p = 0.0047$; elderly with OD: 5.19 ± 1.39 mA, $p = 0.049$) (Fig. 3a). When considering both older groups together (healthy elderly and elderly with OD), pharyngeal sensory threshold was also higher in those with reduced concentration of SP in saliva. (Fig. 3a). In contrast, the differences between the pharyngeal sensory threshold in patients with low CGRP concentration compared to those with high CGRP did not reach statistical significance when analyzed by group or when considering all both older groups together (Fig. 3b).

Discussion

The aim of this study was to assess the relation between salivary SP and CGRP concentration and pharyngeal sensitivity in order to describe a peripheral biomarker in saliva to monitor pharyngeal sensory function in patients with OD and to identify potential "responders" to neurostimulation sensory treatments. The main results concur with our hypothesis as we have clearly observed that the salivary concentration of SP and CGRP were reduced and inversely correlate with pharyngeal sensory threshold, but only SP identified thosepatients with higher pharyngeal sensory threshold. We also observed that in addition to their pharyngeal sensory deficits, elderly with OD patients present a severe delay in the swallow motor responses involved in airway protection, with sarcopenia and hydropenia as main contributors to muscular dysfunction.

Participants in our study were classified in three groups (healthy volunteers, healthy elderly, elderly with OD) showing similar distribution between genders. Older patients in both groups show quite preserved functional and nutritional status and low number of comorbidities. However, our bioimpedance results in healthy elderly and elderly with OD showed increased fat mass and reduced lean, muscular mass, and cellular mass. Both older groups also showed a reduction in the intracellular water compared to healthy volunteers indicating that they had hypertonic dehydration. This hypertonic dehydration might be caused by decreased water intake due to multiple factors, such as reduced thirst sensation and side effects of some drugs, and also OD. This hyperosmotic stress causes cell dehydration which alters different physiological processes, and further contributes to frailty and functional decline in older people [33]. In addition,

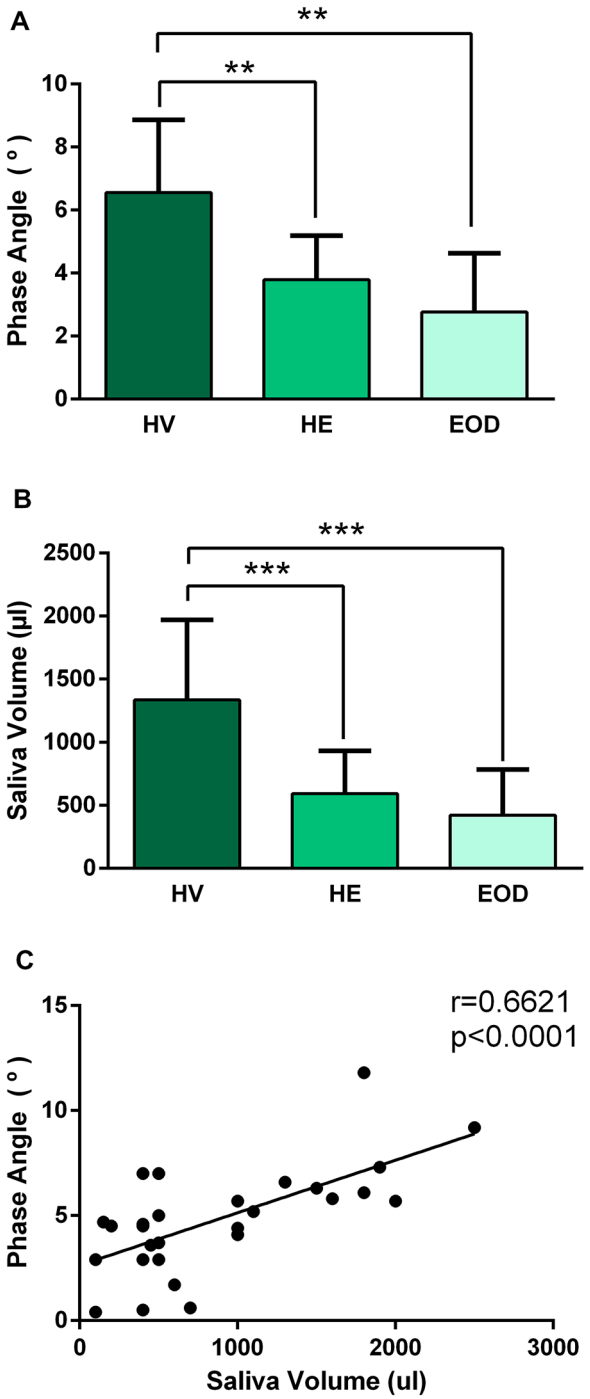

Fig. 1 Bioimpedance results and saliva volume in the three groups: **a** Phase angle, **b** Saliva volume, **c** Correlation between phase angle and saliva volume. *HV* healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia. ° degree, *µl* microliter, $^{**}p < 0.01$, $^{***}p < 0.001$

cell dehydration compromises the integrity of the plasmatic membrane, which alters its capacity to separate charges of the bioimpedance electric current and causes the reduction of the phase angle, as seen in our results. Phase angle is also related to the quality of the muscle function in older adults [34, 35]. Although there is no gold standard for the study of hydration status [36], our study shows a significant reduction in intracellular water and muscle mass in elderly with OD confirming both "sarcopenic" and "hydropenic" pathophysiological elements in the weak muscular swallow response of our older participants. In addition, healthy elderly and elderly with OD showed a reduction in the volume of saliva, with a strong significant positive correlation with phase angle. These results clearly show that the dehydration observed in our patients reduces saliva secretion and this might also contribute to OD [37]. These results match with a previous study of our group where patients with chronic OD showed high prevalence of sarcopenic malnutrition and intracellular dehydration [38] clearly showing the effect of OD on body homeostasis in older people and the involvement of these two elements in the pathophysiology of "sarcopenic" and "hydropenic" dysphagia.

We assessed swallowing function through VFS, showing that the older patients included in the elderly with OD group had high prevalence of efficacy and safety impairments, a mean maximum PAS of 4.38 ± 0.77 and delayed time to LVC and UESO. As shown previously, a delayed swallow response, specifically the time to LVC, is related to impaired safety of swallow and penetrations and aspirations into the airway [4]. In addition, our patients showed a significant decrease in muscle mass. It is already known that there are age-related changes in the swallowing muscles, including tongue and cervical muscles, which are also related to OD and defined as "sarcopenic dysphagia" [39–42]. This phenomenon could explain the high prevalence of oral and pharyngeal residue in both older groups in our study. It is important to note that dehydration and sarcopenia are not only a cause but also a consequence of OD due to the secondary malnutrition and low fluid intake [41].

Previous studies from our group showed that older patients with OD presented an impairment in the oropharyngeal sensory pathway and impairments in the cortical sensory activation that was reflected in the lengthening of the latency and the reduction of the amplitude of the characteristic peaks of the PSEPs [5]. The aging process leads to a reduction of pharyngeal sensitivity assessed with the pharyngeal sensory threshold to electrical stimulation, which is significant in older patients with OD. Up to 35.71% of patients classified in the elderly with OD presented a pharyngeal sensory threshold above the reference interval and showing an impaired pharyngeal sensitivity. The loss of sensory function correlates with peripheral neurodegeneration and a reduction of the oropharyngeal mechanoreceptors in an aging animal model, altering chewing and swallowing functions as a consequence [6]. In human adults, a reduction of the number of small myelinated fibers of the SLN has also

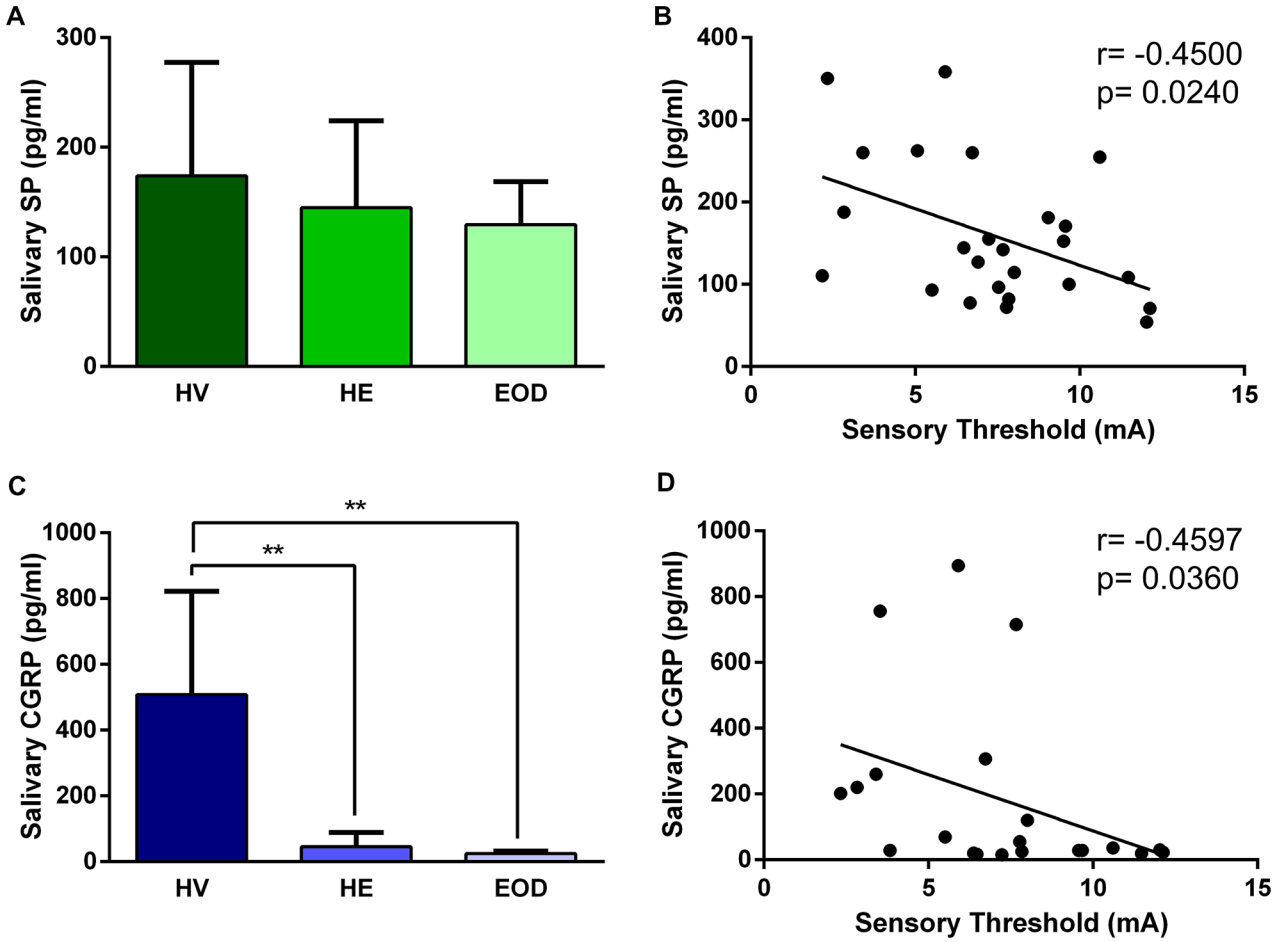

Fig. 2 Salivary neuropeptides and their correlation with pharyngeal sensory threshold. **a** Salivary SP concentration; **b** Correlation between pharyngeal sensory threshold and salivary SP; **c** Salivary CGRP concentration; **d** Correlation between pharyngeal sensory threshold and salivary CGRP. *HV* healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia, *mA* milliampere, *pg/ml* picogram/milliliter $^{**}p < 0.01$

been described [7]. In addition, our group also described a correlation between the latency of the N1 peak of the PSEPs and the time to LVC in older patients with OD [23], suggesting that there is a relationship between the impairment of the pharyngeal sensory function and the slow biomechanics of the airway protection mechanisms (time to LVC). Our present study adds a potential biomarker for this sensory dysfunction as salivary SP has been found to be reduced in this population.

We have also previously described the localization of the TRP and ASIC receptors in the human oropharynx: TRPM8 and ASIC3 are found on submucosal sensory nerves in the human oropharynx; and that TRPV1 and TRPA1 are widely expressed with two distinct patterns: TRPV1 was localized at epithelial cells and nociceptive fibers, in contrast TRPA1 was localized below the basal lamina [8, 9]. We believe that the activation of all these receptors with chemical and thermal bolus stimuli might cause the secretion of SP and CGRP to saliva. Some studies have already shown an increase in the concentration of SP and CGRP in saliva after intrapharyngeal sensory electrical stimulation or capsaicin [19–21]. In addition, our group showed that patients with OD had lower basal concentration of SP than healthy participants of the same age and sex [17], and studies showed that patients with OD with less salivary SP had a lower spontaneous swallowing frequency [32]. In the present study we observed that concentrations of both SP and CGRP in saliva moderately but significantly correlated with the pharyngeal sensory threshold. This correlation has allowed us to divide the population according to the salivary SP and CGRP concentrations they present, showing that those patients with low concentrations of SP have a higher sensory threshold and vice versa. This was not the case for CGRP, which was severely reduced in all older patients and did not allow us to

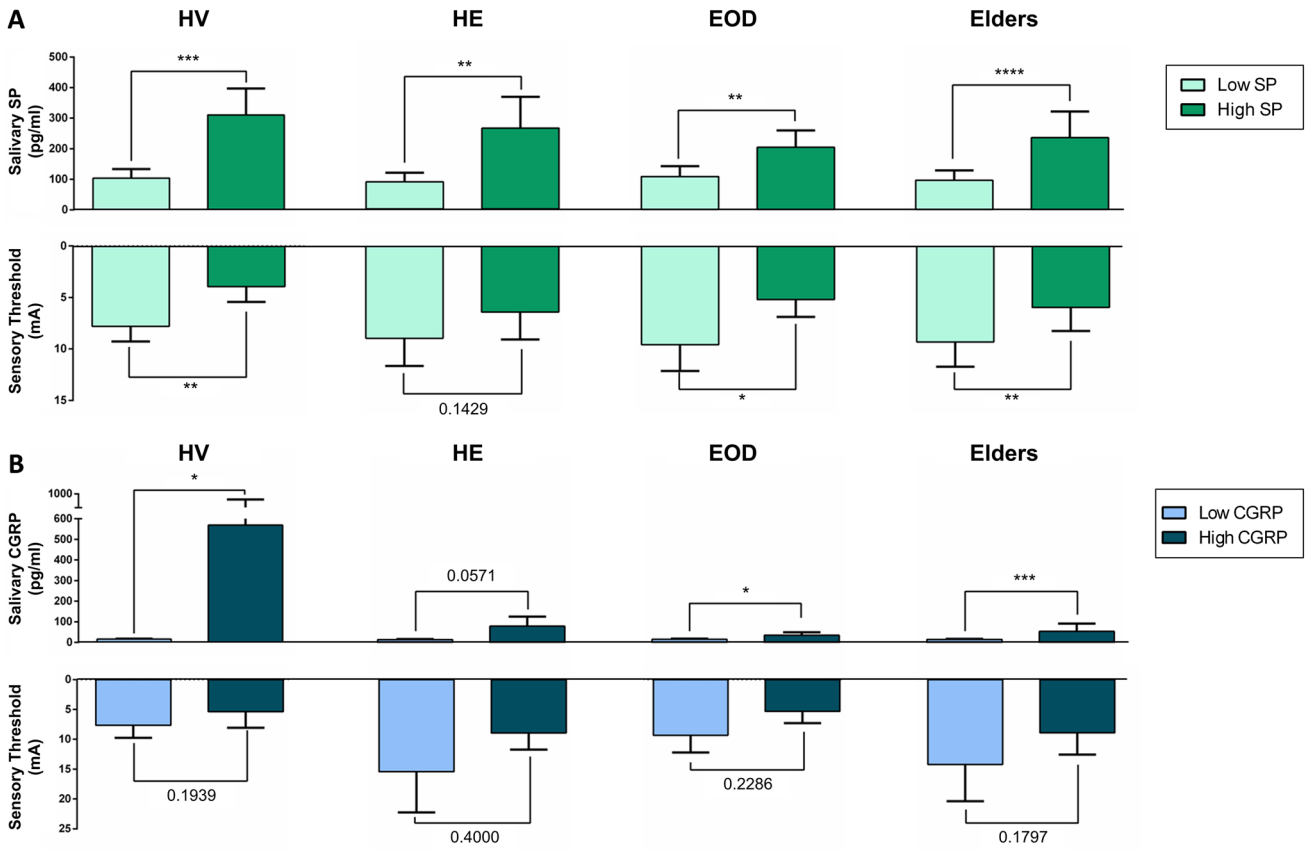

Fig. 3 Division of the groups according to salivary SP and CGRP concentration. **a** Concentration of salivary SP and pharyngeal sensory threshold in low and high SP groups, **b** Concentration of salivary SP and pharyngeal sensory threshold in low and high CGRP groups. *HV* healthy volunteers, *HE* elders without oropharyngeal dysphagia, *EOD* elders with oropharyngeal dysphagia, *pg/ml* picogram/milliliter, *mA* milliampere.*$p<0.05$, **$p<0.01$, ***$p<0.001$

discriminate the impairment of pharyngeal sensitivity based on its concentration in saliva. Therefore we propose salivary SP as a peripheral biomarker for oropharyngeal sensory impairment. We have demonstrated the therapeutic effect on swallowing function of different peripheral and central sensory stimulation strategies in several studies [22–25, 27]. The dynamics of the concentration of SP in saliva before and after the stimuli would indicate which patients respond or not to peripheral sensory neurostimulation treatments.

This study has some limitations. Our sample is small and only includes independently-living older people with mild OD. Further studies are needed to know if this alteration in salivary SP and CGRP is observed in older patients with more severe forms of OD and in patients with OD due to other causes such as stroke or neurodegenerative disease. In addition, studies following sensory stimulation should also demonstrate whether there is an increase in the level of these peptides in the patients that "respond" to these strategies.

In conclusion, this study shows that older people, especially the elderly with OD, have "sarcopenic", "hydropenic" dysphagia, reduced saliva secretion, reduced salivary SP and CGRP concentration and impaired pharyngeal sensory function with increased pharyngeal sensory threshold. We also demonstrate that there is a correlation between salivary SP and CGRP and pharyngeal sensory threshold in elderly patients with OD, but only SP could discriminate pharyngeal sensory threshold impairment according to its concentration in saliva, suggesting its potential role as a peripheral biomarker of impaired pharyngeal sensitivity in this dysphagic population.

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Author contributions PC designed the research study. NT, OO and SC performed the research. WN performed and analyzed the VFS. NT analyzed the data. NT, OO and PC wrote the article. All the authors reviewed the article and approved the final version.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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Noemí Tomsen MSc

Omar Ortega PhD

Weslania Nascimento PhD

Silvia Carrión MD, PhD

Pere Clavé MD, PhD

Sensory Stimulation Treatments for Oropharyngeal Dysphagia

Daniel Alvarez-Berdugo, Noemí Tomsen, and Pere Clavé

Contents

D. Alvarez-Berdugo • N. Tomsen • P. Clavé (✉)
Gastrointestinal Tract Motility Laboratory, CIBERehd-CSdM, Hospital de Mataró, Mataró, Spain
e-mail: pere.clave@ciberehd.org

Abstract

One of the main causes of dysphagia in older and neurological patients is the impairment on oropharyngeal sensory function. Over the last decade, a better understanding of how sensory stimuli are perceived in the human oropharynx and processed in the CNS has led to several new therapeutic strategies based on the sensory stimulation of the oropharynx in the treatment of dysphagia. The goal of these new strategies is not only to prevent safety impairments during deglutition but to improve the oropharyngeal swallow response to move from compensation to recovery of the swallowing function. This chapter will cover the neurophysiological, anatomical and molecular bases for these sensory stimulation treatments and provide an updated list of therapies that have been validated on patients with dysphagia, including pharmaceutical, physical and electrical stimulation of the oropharynx.

1 Introduction

One of the main causes of dysphagia in older patients and those with stroke and neurodegenerative diseases is the impairment on oropharyngeal sensitivity. Oropharyngeal sensitivity may be reduced by varying mechanisms between these groups, but all are closely related to delayed oropharyngeal swallow response (OSR) and safety impairments.

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Oropharyngeal sensitivity is known to decrease with age, with close correlation between age and sensory threshold for mechanical stimulation (Aviv et al. 1994). This has been explained by the observation that the ageing process reduces the number of small diameter myelinated fibres in the internal superior laryngeal nerve (ISLN) (Mortelliti et al. 1990; Tiago et al. 2007). Electroencephalographic (EEG) studies have also shown how older people present lower amplitude and greater latency in their pharyngeal sensory evoked potentials (PSEP) than young volunteers. This could be due to both a decreased afferent input from the pharynx and disrupted connection within the cortex (Rofes et al. 2017). Pharyngeal sensory loss is even greater in older dysphagia patients; this oropharyngeal sensory impairment is related to increased prevalence of laryngeal vestibule penetrations and aspirations (Aviv et al. 1994; Rofes et al. 2017).

Oropharyngeal sensitivity can be affected by stroke, supratentorial or infratentorial, and stroke patients with decreased pharyngeal sensitivity are at high risk of aspiration, which can lead to aspiration pneumonia (Aviv et al. 1996, 1997). Although the oropharyngeal motor representation in the cortex is asymmetrical and thus only a stroke on the dominant hemisphere affects the motor aspect of swallow response (Hamdy et al. 1997; Singh and Hamdy 2006), the oropharyngeal sensory representation in the cortex can be affected independently of the dominant hemisphere, and oropharyngeal sensitivity can be reduced in the contralateral side of the pharynx (Aviv et al. 1997; Cabib et al. 2017).

Oropharyngeal sensitivity is also impaired in patients with neurodegenerative diseases such as Parkinson's disease (PD), which accounts for a lack of self-awareness of swallowing disorders in these patients (Hammer et al. 2013). Impaired oropharyngeal sensitivity in neurodegenerative diseases is due not only to damage in the central nervous system (CNS) but also to damage in the peripheral sensory nerves innervating the oropharynx (Mu et al. 2013, 2015); Lewy pathology has been found in sensory nerve fibres innervating the oropharynx and larynx, specially in the ISLN of PD patients with dysphagia symptoms. Damage to the peripheral sensory nerves has also been found in other Lewy body disorders, such as Alzheimer's disease and dementia with Lewy bodies (Beach et al. 2010).

Oropharyngeal sensory inputs play a major role in the generation of the swallow response. Decreased oropharyngeal sensitivity described in older, stroke and neurodegenerative disease patients is thus a critical component of the impaired OSR in dysphagia patients that can be and must be targeted to treat oropharyngeal dysphagia.

2 Swallow Neurophysiology

The proper generation of a coordinated OSR requires the interaction and connection of several areas and elements from the CNS and the peripheral nervous system. It also requires structural integrity of the oropharynx and larynx, proper function of 30 pairs of muscles and coordination with the respiratory system (Fig. 1).

Stimuli from the alimentary bolus are perceived by the peripheral sensory receptors during deglutition; this information is sent by the afferent nerves (cranial nerves V, VII, IX and X) to the central pattern generator (CPG) located in the medulla oblongata of the brainstem and to the somatosensory cortex and subcortical structures such as the amygdalae and the basal ganglia (Clavé and Shaker 2015).

The voluntary control of the oral phase and part of the pharyngeal phase of deglutition are conducted in cortical areas of the brain such as the precentral and inferior frontal gyri as well as other adjacent cortical areas of the sylvian fissure and the lateral and precentral cortex (Schindler and Kelly 2002). In healthy individuals, deglutition activates these cortical areas in a bilateral but asymmetric fashion, which implies the existence of a dominant hemisphere (Hamdy et al. 1996; Teismann et al. 2011).

The OSR of the pharyngeal phase of deglutition is produced in the CPG found in the medulla oblongata of the brainstem. The CPG is composed of two well-communicated groups of interneurons: the dorsal swallowing group (DSG) and

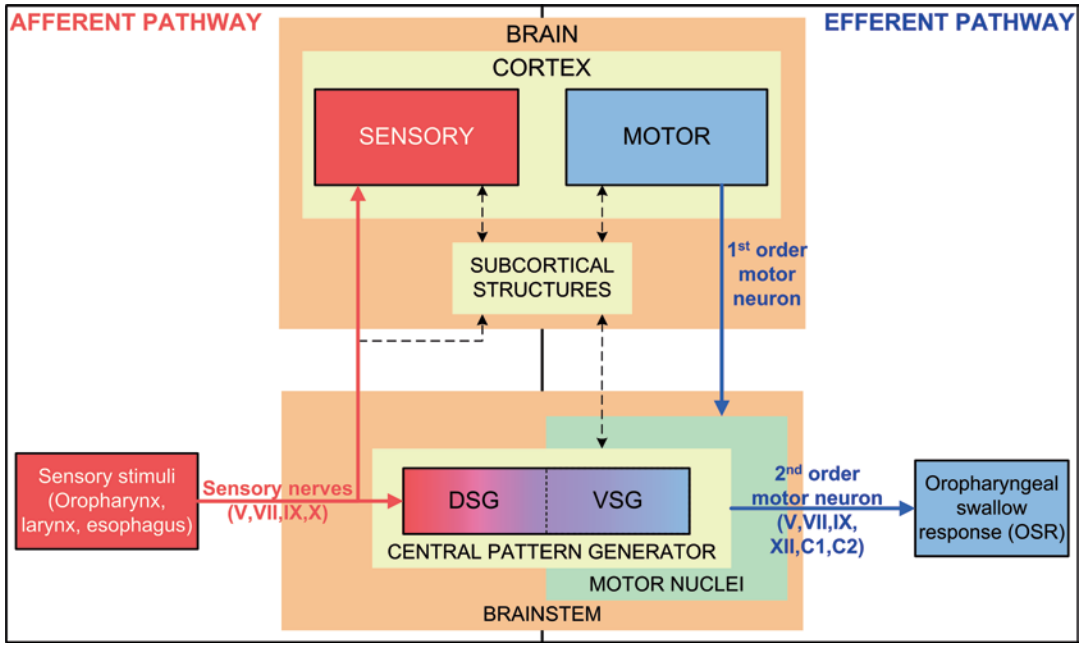

Fig. 1 Swallow neurophysiology. *DSG* dorsal swallowing group; *VSG* ventral swallowing group

the ventral swallowing group (VSG). The DSG is found in the nucleus tractus solitarius within the medulla oblongata and integrates the afferent information from the peripheral nerves and the modulating signals coming from cortical and subcortical structures to generate the swallowing motor pattern. The VSG is found in the ventrolateral aspect of the medulla oblongata above the nucleus ambiguus and, upon activation by the DSG, distributes the swallowing motor pattern among the different motor nuclei (Jean 2001).

The motor nuclei controlling the deglutition muscles are found in the pons of the brainstem (trigeminal motor nucleus and facial nucleus), in the medulla oblongata (nucleus ambiguus and hypoglossal nucleus) and in the cervical spinal cord (C1–C2). Motor neurons innervating oropharyngeal muscles project from these nuclei through the cranial nerves V, VII, IX, X and XII and the cervical spinal nerves (C1, C2 and C3) composing the cervical plexus.

The muscles that participate in deglutition are the masticatory, the lingual, the soft palate, the pharyngeal, the laryngeal, the suprahyoid and the infrahyoid muscles. During the oral phase, the masticatory muscles are innervated by the cranial nerves V and VII, and the lingual muscles are innervated by the cranial nerve XII. During the pharyngeal phase, the soft palate, the pharyngeal and the extrinsic laryngeal muscles are innervated by the pharyngeal plexus composed by the cranial nerves IX and X, the suprahyoid muscles are innervated by the cranial nerves V, VII and XII, the intrinsic laryngeal muscles are innervated by the recurrent laryngeal nerve (cranial nerve X) and the infrahyoid muscles are innervated by the cervical plexus.

3 Sensory Innervation of the Oropharynx and Larynx

The human oropharynx and larynx are innervated by afferent fibres of the lingual branch of the cranial nerves V and IX, the chorda tympani of the cranial nerve VII, the pharyngeal branch of the cranial nerves IX and X and the laryngeal branch of the cranial nerve X (Fig. 2).

The tongue is innervated by afferent fibres of the lingual branches of the cranial nerves V, VII and IX (L-IX). The base of the tongue and the

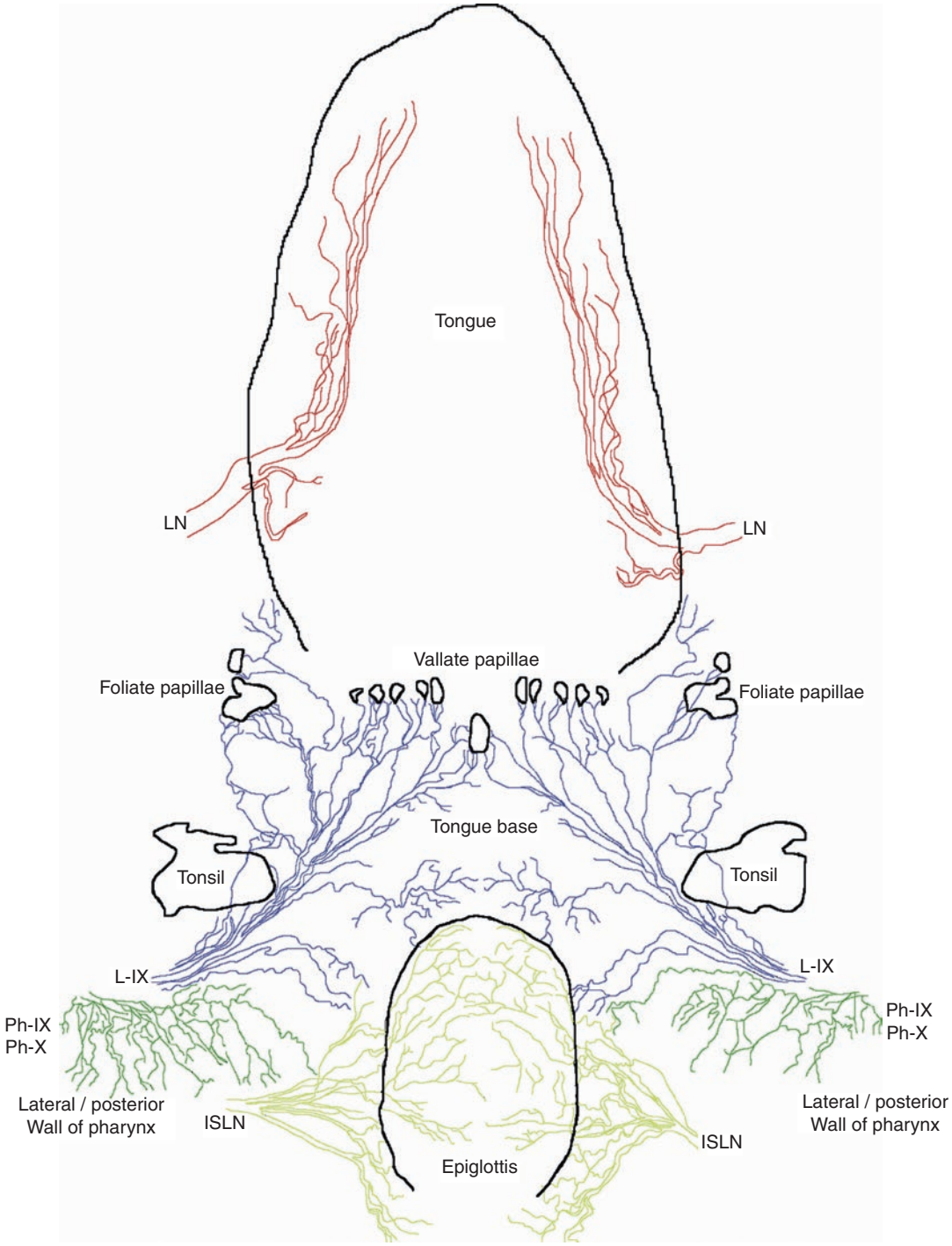

Fig. 2 Distribution of the oropharyngeal sensory innervation. Schematic representation of the distribution of the sensory branches of CN V, VII, IX and X innervating the oropharyngeal mucosa. *LN* lingual nerve, *ISLN* internal superior laryngeal nerve. Based on the Sihler's stain images from (Sanders and Mu 1998; Mu and Sanders 2000; Zur et al. 2004). Reproduced from Alvarez-Berdugo et al. (2016a)

circumvallate and foliate papillae are innervated by two of the four major subdivisions of the L-IX. The subdivision of the L-IX that innervates the base of the tongue mucosa is divided into tertiary branches and twigs that run towards the medial axis of the tongue where they meet with the fibres coming from the opposite side. The subdivision of the L-IX that innervates the papillae is divided in lateral and medial branches; lateral branches innervate the foliate papillae and some of the lateral circumvallate papillae, medial branches ipsilaterally innervate most of the circumvallate papillae. The central circumvallate papilla is innervated by fibres coming from medial branches from both sides (Mu and Sanders 2000). The two anterior thirds of the tongue are innervated by the lingual nerve (LN) composed of the lingual branch of the cranial nerve V and some afferent fibres from the chorda tympani. The LN penetrates the tongue anterior to the circumvallate papillae and subdivides into medial and lateral branches; the medial branches innervate the ventrolateral mucosa of the tongue, and the lateral branches innervate the lateral part of the tongue and its tip (Zur et al. 2004).

The oropharyngeal mucosa is innervated by afferent fibres of the lingual (L-IX) and pharyngeal (Ph-IX) branches of the cranial nerve IX and the pharyngeal branch of the cranial nerve X (Ph-X). The mucosa of the tonsil and peritonsillar area and the lingual surface of the epiglottis are innervated by two of the four subdivisions of the L-IX. The subdivision of the L-IX that innervates the tonsil and peritonsillar area is split into two or three tertiary branches that surround the tonsil. The subdivision of the L-IX that innervates the epiglottis is also split into two or three tertiary branches; one of them innervates the mucosa of the lingual surface of the epiglottis, and the others form anastomosis with afferent fibres from the laryngeal sensory branch of the cranial nerve X, the internal superior laryngeal nerve (ISLN). The lateral and posterior walls of the pharynx, including the pharyngopalatine arch, are innervated by the three subdivisions of the Ph-IX together with the afferent fibres of the Ph-X; all these afferent fibres form a dense nervous plexus in the mucosa of this region (Mu and Sanders 2000).

The mucosa of the hypopharynx and larynx is innervated by the ISLN, which penetrates the thyrohyoid membrane and splits into three branches. The laryngeal surface of the epiglottis is innervated by the superior branch of the ISLN, which penetrates the epiglottis through the aryepiglottic fold and subdivides into twigs that form a dense network with the fibres arriving from both sides. The aryepiglottic fold, the laryngeal vestibule and the vocal folds are innervated by the middle branch of the ISLN, which splits into interconnected twigs that form a network under the mucosa. The other parts of the hypopharynx are innervated by the inferior branch of the ISLN, the one with greatest diameter and most complex distribution: the superior subdivision of which innervates the arytenoid cartilage, the middle subdivision innervates the region posterior to the arytenoids and the interarytenoid muscles and the inferior subdivision innervates the mucosa over the anterior wall of the hypopharynx (Yatake and Hiroto 1968; Sanders and Mu 1998).

4 Sensory Structures of the Oropharynx Involved in Swallow Function

The afferent fibres innervating the human oropharynx perceive chemical (taste and pungency), thermal and mechanical stimuli. Mechanical, thermal, chemical (excluding taste) and painful stimuli are perceived in the oropharynx by the corresponding branches of the cranial nerves V, IX and X. Taste is perceived by the afferent fibres of the chorda tympani (cranial nerve VII) that innervate the fungiform papillae of the anterior two thirds of the tongue and by the afferent fibres of the L-IX branch that innervate the circumvallate and foliate papillae of the tongue base. The sensory structures that perceive all these stimuli are the Meissner's corpuscles, the Merkel cells, the Pacini corpuscles, the Krause bulbs, the free nerve endings and the taste buds (Alvarez-Berdugo et al. 2016a) (Fig. 3).

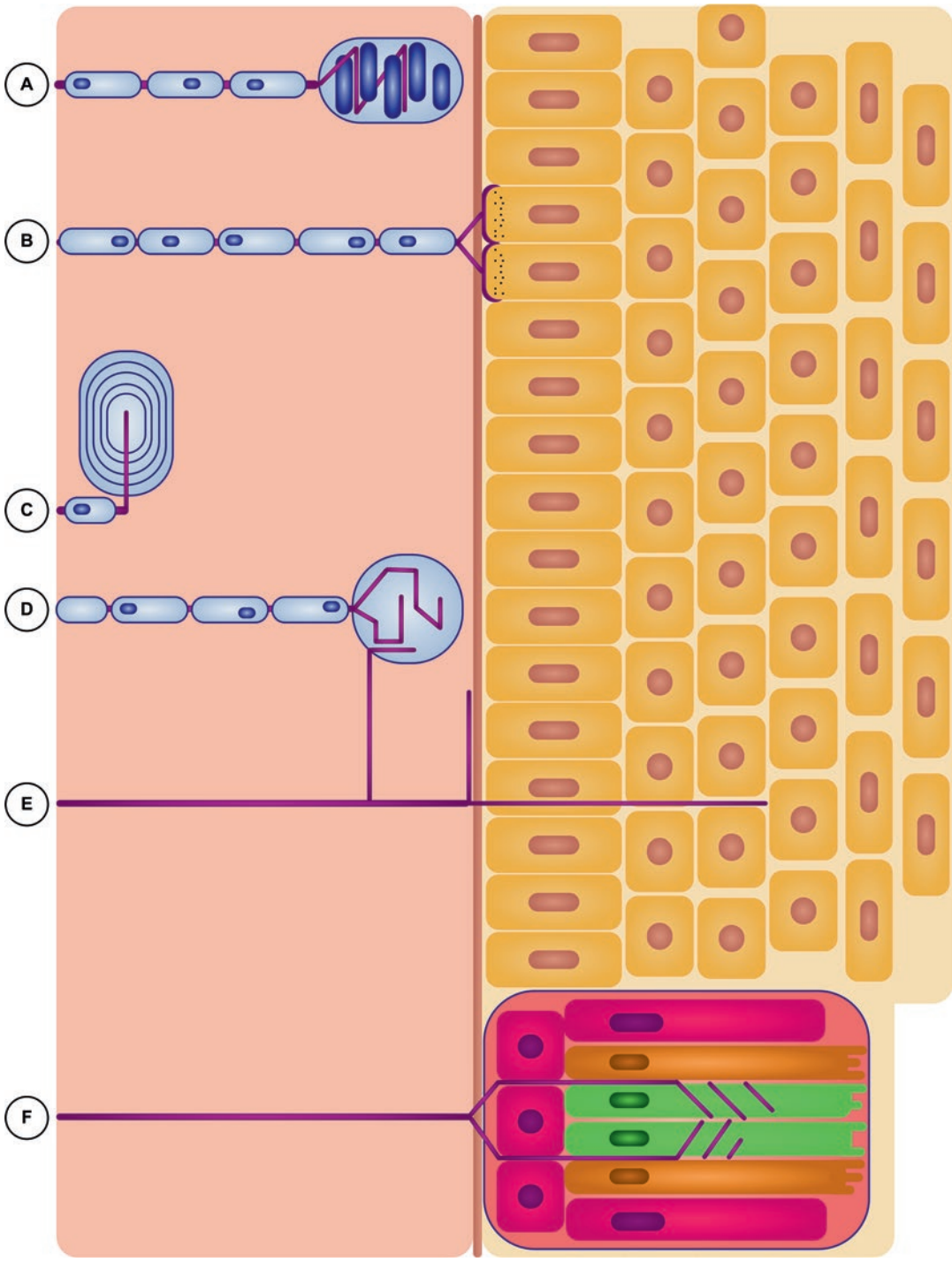

Fig. 3 Specialized sensory terminals found in the mucosa. Schematic representation of the specialized sensory terminals perceiving mechanical, thermal and chemical stimuli in the oropharyngeal mucosa. (**a**) Meissner's corpuscles, (**b**) Merkel cells, (**c**) Pacini corpuscle, (**d**) Krause bulb, (**e**) free nerve ending, (**f**) taste bud. Reproduced from Alvarez-Berdugo et al. (2016a)

4.1 Meissner's Corpuscles

Meissner's corpuscles (Fig. 3a) perceive painless light mechanical stimuli. They are found in groups within the papillae disposed in different directions. They are supplied by one to three myelinated nerve fibres that lose their myelin sheath on entering the corpuscle and then divide into branches or form a discoid shape. The nerve endings are covered with several lamellar layers separated by collagen fibre-filled interspaces. The corpuscles are covered with a collagen capsule that keeps them separated from the basal lamina of the epithelium (Watanabe 1982; Watanabe and Yamada 1983, 1985).

4.2 Merkel Cells

Merkel cells (Fig. 3b) perceive the lightest touch without pain. They are found alone or in groups in the basal layer of the epithelium, always bound to the basal lamina. They are innervated by nerve endings that penetrate the basal lamina after losing their myelin sheath and surround the base of these cells. Merkel cells present granules in the basilar end and protrusions of cytoplasm that contact neighbouring epithelial cells at the apical end (Smith 1970; Hashimoto 1972; Toyoshima et al. 1987; Bengoechea et al. 1989).

4.3 Pacini Corpuscles

Pacini corpuscles (Fig. 3c) perceive deep mechanical stimuli without pain. They are found in the deepest layer of the submucosa. They are innervated by a single myelinated nerve end that penetrates the corpuscle in a straight line. Pacini corpuscles are lamellar structures (Munger and Ide 1988; Watanabe 2004).

4.4 Krause Bulbs

Krause bulbs (Fig. 3d) perceive light touch and cold stimuli. They are found within the papillae. They are innervated by one to three myelinated or not myelinated nerve endings, when both kinds of nerve endings penetrate the same bulb they never contact each other. Once the nerve endings have penetrated the bulb, they run in coils and split into several axon terminals. Krause bulbs are covered by a capsule composed of fibroblasts and collagen fibres (Chouchkov 1973; Lawrenson and Ruskell 1991).

4.5 Free Nerve Endings

Free nerve endings (Fig. 3e) perceive painful, chemical and thermal stimuli. They split into branches and reach the basal lamina of the epithelium where, in some cases, the Schwann cells that surround these terminals and the basal lamina establish close contact and become thinner so the axon terminal can penetrate the epithelium. However, intraepithelial sensory fibres are scarce in the oropharyngeal mucosa. Free nerve endings are generally type Aδ and C nerve fibres (Bengoechea et al. 1989; Munger 1965; Chiba et al. 1985).

4.6 Taste Buds

Taste buds (Fig. 3f) distinguish between the five basic tastes: salt, sweet, bitter, sour and umami, the taste elicited by the presence of glutamate and ribonucleotides such as IMP and GMP in the food. They are found on the fungiform papillae of the two anterior thirds of the tongue and the soft palate, in the rifts of the circumvallate papillae and on the foliate papillae of the tongue base. Taste buds are onion-like structures formed by specialized epithelial cells (Stone et al. 1995; Okubo et al. 2009; Roper 1989), which can be classified according to their morphology and function as Type I, II and III. Type I cells are the most abundant (50–75% of the taste bud cells) and provide cellular support to the other cells; they also transduce the salt taste (Chaudhari and Roper 2010; Vandenbeuch et al. 2008). Type II cells transduce the sweet, bitter and umami tastes, depending on the specialized receptor they express (Zhang et al. 2003; Tomchik et al. 2007).

Type III cells establish a synaptic union with the nerve endings that supply the taste buds and transduce salt and sour tastes (Yee et al. 2001; Tomchik et al. 2007). All these taste bud cell types are connected; Type I cells provide support to the perception and synaptic communication of Type II and III cells. Type II cells finely perceive tastes and send signals to Type III cells that will transmit the stimulus information to the nerve endings (Chaudhari and Roper 2010). The nerve fibres supplying the taste buds penetrate the basal lamina of the epithelium and form protrusions that surround Type III cells to establish synapse (Yee et al. 2001; Witt and Reutter 1997).

5 Sensory Receptors of the Oropharynx

Perception of thermal and chemical stimuli is achieved through the presence of molecular receptors expressed in the sensory nerves that innervate the oropharyngeal mucosa. The activation of these receptors will initiate the transmission of a sensory input towards the CNS. The main family of sensory receptors found in the human oropharynx are the transient receptor potential (TRP) channels which perceive a wide spectrum of temperatures and chemical substances (Alvarez-Berdugo et al. 2016a) (Table 1).

5.1 TRPV1

The transient receptor potential channel subfamily V member 1 (TRPV1) was the first TRP receptor identified. It was characterized as the vanilloid receptor from a cDNA library expressed in mammalian cells (Caterina et al. 1997). This receptor is mainly expressed in the afferent Aδ and C fibres, together with the pro-inflammatory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP). Furthermore, its expression has been found in multiple human tissues, both in nerve (such as dorsal root ganglia and trigeminal ganglia) and non-nerve tissue (such as epithelial cells of the urinary bladder and the skin) (Cortright et al. 2001; Ugawa et al. 2005; Denda et al. 2001; Shabir et al. 2013).

TRPV1 was found in the plasma membrane of the oropharyngeal and epiglottis lingual surface mucosa epithelial cells, with stronger signal in the basal and intermediate layer cells than in the more superficial layer cells of the epithelium. It

Table 1 Natural agonists and physical activators of TRPV1, TRPA1 and TRPM8

TRPV1	TRPA1	TRPM8
Temperature (≥43 °C)	Temperature (≤17 °C)	Temperature (≤25 °C)
Acidic pH	1,4-Dihydropyridines	Eucalyptol (eucalyptus)
2-APB	Allicin (garlic)	Geraniol (geranium, lemon)
Allicin (garlic)	Allyl isothiocyanate (mustard)	Icilin
Anandamide	Bradykinin	Linalool (tejpat)
Camphor (camphor laurel)	Cannabichromene (cannabis)	Menthol (peppermint)
Cannabidiol (cannabis)	Cannabidiol (cannabis)	
Cannabigerol (cannabis)	Cannabinol (cannabis)	
Capsaicin (chilli peppers)	Capsiate (chilli peppers)	
Eugenol (cloves)	Cinnamaldehyde (cinnamon)	
Gingerol (ginger)	Curcumin (turmeric)	
Hydrogen sulphide	Eugenol (cloves)	
Nitric oxide	Gingerol (ginger)	
Piperine (black pepper)	Hydrogen sulphide	
Polygodial (Dorrigo pepper)	Icilin	
	Nitric oxide	
	Tetrahydrocannabinol (cannabis)	

Reproduced from Alvarez-Berdugo et al. (2016a)

was also found in the nociceptive Aδ fibres innervating the oropharyngeal mucosa and in polymorphonuclear leukocytes in the submucosa, near the basal lamina. In the lingual mucosa, TRPV1 was mainly found on the membranes of the stratum basale and stratum spinosum cells of the filiform papillae epithelium; similarly to the oropharyngeal mucosa, TRPV1 immunoreactivity was weaker in the more superficial layers (stratum granulosum and stratum corneum) (Alvarez-Berdugo et al. 2016b).

The main function of TRPV1 channels is to open upon perception of harmful stimuli. This receptor is activated by harmful temperatures (>43 °C), acid solutions (pH < 5.5) and several endogenous and exogenous chemical compounds, such as capsaicin, one of the better known chemical agonists of TRPV1 (Szallasi et al. 2007).

TRPV1 ion channels are homotetramers and each subunit has six transmembrane domains with the C-terminal and N-terminal in the cytosolic space. The pore of the channel is formed by two of these transmembrane domains, and the sensory function is performed by the other four (vanilloid binding site). The cytosolic domains allow specific protein-protein interactions due to the presence of four to six ankyrin repeats. Among these interactions, calmodulin is the most important, regulating the function of the channel according to the intracellular calcium levels (Kedei et al. 2001; Mosavi et al. 2002; Rosenbaum et al. 2004). There are other modulators that interact with the cytosolic domains of TRPV1, such as PIP2, which inhibits the channel activation, and PKA and PKC phosphatases, which sensitize the channel by phosphorylating their target amino acids (Rathee et al. 2002; Rosenbaum and Simon 2007).

The mechanism through which TRPV1 is activated depends on the stimulation agent. While high temperatures seem to affect several domains of the channel, chemical agonists interact with the vanilloid binding site (Voets 2014):

- Acid: The opening of the channel depends on the oropharyngeal acidity. When the pH is below 6, the pore opens due to the action of H+ ions on the extracellular domain (Glu-648, Val-538 and Thr-633). When the pH is between 6 and 7, H+ ions act on the Glu-600 reducing the concentration threshold for other agonists to activate the channel (Geppetti and Trevisani 2004; Nilius et al. 2007).
- Pungency: The activation of TRPV1 by capsaicin and other vanilloids might increase the sensory input to the brainstem and cortical areas, activating the CPG and eliciting the swallow response. In addition, the release of neuropeptides, such as SP and CGRP, may produce local effects through paracrine and autocrine mechanisms. SP is released after the activation of TRPV1 by its agonists; SP then mediates the phosphorylation of TRPV1 via PKC and thus sensitizes the receptor and decreases its activation threshold. The release of SP into the larynx, either through direct action on sensory terminal nerves or through the action of pro-inflammatory substances, can also sensitize primary sensory neurons and thus facilitate the motor swallow response. SP also plays a major role in the cough reflex (Nakagawa et al. 1995; Rofes et al. 2014a).

5.2 TRPA1

Following the identification of the TRPV1, other nociceptive receptors were identified, among them the transient receptor potential channel subfamily A member 1 (TRPA1), formerly called ANKTM1, as the noxious cold stimuli receptor. TRPA1 is expressed in a subpopulation of C-fibre sensory neurons from the nodose, trigeminal and dorsal root ganglia that also expresses TRPV1, as well as SP and CGRP neuropeptides (Story et al. 2003; Bautista et al. 2005; Kobayashi et al. 2005; García-Añoveros and Duggan 2007). TRPA1 expression has also been found in fibroblasts and epithelial cells of the skin and the bronchial walls, as well as in ear ciliated cells (Corey et al. 2004; Atoyan et al. 2009; Mukhopadhyay et al. 2011).

Within the oropharynx, TRPA1 can be found below the basal lamina, in sensory nerve fibres that are just below the epithelium. It is also found within the epithelium, either in the nerve fibres

that penetrate it or in the Langerhans cells of the stratum spinosum of the lingual mucosa or the intermediate layer of the pharyngeal and lingual surface of the epiglottis mucosa. TRPA1 can also be found in the sensory fibres that innervate the blood vessels that irrigate the submucosa (Alvarez-Berdugo et al. 2016a, b).

TRPA1 is the receptor of noxious cold temperatures (<18 °C) and natural and synthetic irritant substances such as allyl isocyanate (mustard), cinnamaldehyde (cinnamon), piperin (black pepper), allicin and allyl disulphide (garlic), and it can also be activated by low concentration of menthol (Bandell et al. 2004; Karashima et al. 2007). In addition to chemical and thermal perception, TRPA1 is also involved in mechanoreception in ear ciliate cells (Sotomayor et al. 2005).

The TRPA1 ion channels are homotetrameric and each subunit has six transmembrane domains. The pore of the channel is formed by one of these transmembrane domains from each subunit. What distinguishes TRPA1 from other TRP receptors is its N-terminal end, a cytosolic domain in the shape of a spring due to its 17 ankyrin repeats. It is believed that this structure allows mechanoreceptor function in ear ciliate cells (Sotomayor et al. 2005).

5.3 TRPM8

The transient receptor potential subfamily M member 8 (TRPM8) is another important sensory receptor related with thermal and chemical perception in the human oropharynx. At the neuronal level, TRPM8 expression is concentrated in a subpopulation of peripheral sensory neurons of small diameter, in which there is no co-expression with the TRPV1 receptor or CGRP (Peier et al. 2002). Outside the nervous system, the presence of TRPM8 has been described in different types of solid tumours and has an important role in the survival, proliferation and invasion of tumour cells (Yee 2015).

Within the oropharynx, TRPM8 is mainly located in the nerve fibres that innervate the lingual, oropharyngeal and epiglottis lingual surface mucosa. These nerve fibres are generally located below the epithelium, either in nerve bundles or individually, although they can also trespass the basal lamina and penetrate the deeper layers of the epithelium. TRPM8 has also been found in corpuscular structures under the epithelium and on nerve fibres that innervate the blood vessels that irrigate the submucosa.

TRPM8 is activated by non-harmful cold stimuli (15–30 °C) and chemical substances with a refreshing effect such as menthol, icyline and eucalyptol.

TRPM8 structure is similar to other TRP receptors, but its cytosolic domains do not present ankyrin repeats. Instead it has major homology regions (MHR), which allow the formation of the channel by the union of different subunits of the receptor (Phelps and Gaudet 2007).

5.4 Other TRP

In addition to TRPV1, TRPA1 and TRPM8, there are other TRP receptors involved in the sensory perception of other kinds of stimuli. For instance, TRPV2 is activated by extremely hot temperatures (>52 °C), and TRPV3 perceives temperature changes within the physiologic range (22–40 °C). Furthermore, osmotic changes are perceived by TRPV4 which, along with TRPC1 and TRPC6, can also perceive harmful mechanical stimuli. Little is known about the presence of these receptors in the human oropharynx, however, TRPV2 has been found in rat pharyngeal mucosa, soft palate, epiglottis and larynx. Like TRPV1, TRPV3 has also been found in mouse keratinocytes and neurons. TRPV4 has been found in trachea and salivary glands. (Caterina et al. 1999; Delany et al. 2001; Peier 2002; Xu et al. 2002, 2006; Alessandri-Haber et al. 2003, 2009; Chung et al. 2004; Sasaki et al. 2013).

5.5 ASICs

ASICs are another family of sensory receptors. ASIC family receptors perceive acidic and mechanical stimuli and have been localized in the taste

buds and in the digestive tract (Page et al. 2005; Huque et al. 2009). Among this family of receptors, ASIC3 is thought to be one of the main receptors to perceive acidic stimuli in the oropharynx.

Within the oropharynx, ASIC3 is primarily found in the sensory nerve fibres that innervate the lingual, oropharyngeal and epiglottis lingual surface mucosa. These nerve fibres can be found below the basal lamina, running parallel to the epithelium or innervating the blood vessels that irrigate the submucosa.

ASICs form heteromeric channels between the members of the same family of receptors, which can differentiate between ranges of acidity (Huque et al. 2009). ASICs respond to lower pH ranges than TRPV1 and rapidly inactivate inward Na + currents (Leffler et al. 2006).

6 Sensory Stimulation Treatments

The new stimulation treatments for OD target the reduced oropharyngeal sensitivity in OD patients. The development of these new strategies is possible thanks to greater understanding of swallow neurophysiology and sensory perception in the oropharynx. Most of these strategies aim to activate sensory receptors to increase the oropharyngeal sensory input to the CNS and thus improve the oropharyngeal swallow response. During the last decade, several groups have tested therapeutic strategies based on sensory stimulation in OD patients.

6.1 Mechanical and Thermal Stimulation

Mechanical and thermal stimuli have been used in the past to find the pharyngeal sensory areas that trigger deglutition and to measure the loss of pharyngeal sensitivity in OD patients and populations at risk of dysphagia (Pommerenke 1927; Aviv et al. 1994, 1996, 1997). They have also been used in acute treatments of neurogenic patients with OD to improve swallowing physiology. For instance, cold and mechanical stimulation of the glossopharyngeal innervated regions significantly reduced total transit time in neurogenic OD patients (de Lama Lazzara et al. 1986). These results were reproduced with a metallic instrument that provided 0–3 °C tactile and cold stimuli in the same area in patients with idiopathic Parkinson's disease and OD (Regan et al. 2010). Mechanical stimuli with air pulses have been used as a treatment for stroke patients with OD and were found to improve the swallowing rate (Theurer et al. 2013). Mechanical thermal stimulation of the oropharynx improves the OSR of neurogenic OD patients by increasing the sensorimotor cortical activation (Teismann et al. 2009).

6.2 Chemical and Pharmacological Stimulation

- Acid: One of the first approaches to use chemical sensory stimulation as a therapeutic strategy to treat OD was with sour boluses (50% v/v citric acid from lemon juice). It was found to reduce the prevalence of aspirations by shortening the pharyngeal swallow delay in patients with neurogenic OD, but the high concentration of citric acid was not well tolerated (Logemann et al. 1995). Later studies proved that an equivalent concentration of citric acid (2.7% w/v) achieved a similar therapeutic effect, but mixing citric acid and sucrose (1.11% and 8% w/v, respectively) did not significantly improve OD symptoms or swallow response parameters (Pelletier and Lawless 2003). Finally, stimulating with both cold (2–8 °C) and acid stimuli (citric acid from lemon juice) reduced the pharyngeal swallowing time in stroke patients (Hamdy et al. 2003; Cola et al. 2012).
- TRP agonists: Another early attempt to use chemical stimulation of the swallow reflex was with capsaicin (10^{-6}–10^{-9} M), the pungent substance found in red chilli peppers. Drops were directly instilled into the pharynx and reduced the swallow reflex latency in stroke and dementia patients in a dose-dependent manner (Ebihara et al. 1993). Some years later, capsaicin and other pungent

molecules such as piperine, the pungent substance found in black pepper, were used on patients with OD caused by either age, stroke or neurodegenerative diseases. In both in acute and subacute clinical trials, capsaicin and capsaicinoids (10^{-5} M) were administered in the bolus to OD patients and reduced both safety and efficacy impairment prevalence by shortening the laryngeal vestibule closure time and increasing the bolus velocity (Ebihara et al. 2005; Rofes et al. 2013a; Ortega et al. 2016). Olfactory stimulation with black pepper oil and oral stimulation with piperine within the bolus (10^{-4}–10^{-3} M) also significantly reduced safety impairment prevalence in OD patients (Ebihara et al. 2006a, Rofes et al. 2014a, b), and, when applied during a long period of time (30-day study), it even enhanced neural plasticity in the left insular cortex (Ebihara et al. 2006a). Finally, the use of menthol, either instilled into the pharynx (10^{-4}–10^{-2} M) or mixed with the bolus (10^{-3}–10^{-2} M), reduced the prevalence of safety impairment by improving the swallow response (Ebihara et al. 2006b; Alvarez-Berdugo et al. 2017). However, compared to the therapeutic effect of capsaicinoids or piperine, menthol is the least effective of these agonists (Alvarez-Berdugo et al. 2017). A combination of all these treatments was used in OD patients admitted to hospital with recurrent pneumonia prior to reintroducing oral feeding. Starting with 3 days of olfactory stimulation with black pepper oil once pneumonia had been cured, capsaicin troches were then administered for 5 days, and, finally, a menthol-flavoured jelly was fed to patients prior to oral feeding introduced in a step-by-step manner (Ebihara et al. 2010). Although acute clinical trials have been done as proof of concept to assess the therapeutic effect of these chemical and pharmacological strategies, more mid- and long-term studies will be needed to assess the long-term effect of a chronic treatment using these strategies. Long-term studies are also needed to confirm the effect of these strategies on the CNS.

- Carbonation: Some clinical studies have tested the use of carbonated fluids (obtained by mixing citric acid and sodium bicarbonate) in neurogenic OD patients; analysing deglutition with VFS, these studies proved that carbonated fluids reduce the prevalence of laryngeal vestibule penetrations and pharyngeal residues by improving the OSR (Bülow et al. 2003; Sdravou et al. 2012; Larsson et al. 2017). Studies on healthy volunteers have shown that stimulation with carbonated liquids increases corticobulbar excitability, which would explain the OSR improvement in OD patients (Elshukri et al. 2016).

6.3 Electrical Stimulation

The swallow response can be elicited by direct electrical stimulation of the Ph-IX nerve and the ISLN. On the other hand, electrical stimulation of the L-IX nerve inhibits swallow, and electrical stimulation of the Ph-X nerve has no effect on swallow response (Kitagawa et al. 2002). Voltage-dependent channels of the sensory neurons of these nerves might be activated by sensory electrical stimulation and thus the input signal conducted to superior areas of the CNS.

Electrical stimulation therapies are currently being performed in two different modalities: through intrapharyngeal electrical stimulation or through transcutaneous electrical stimulation.

- *Intrapharyngeal electrical stimulation*: Intrapharyngeal electrical stimulation applies electrical stimuli on the pharynx using intrapharyngeal electrodes. The application of 5 Hz electrical stimuli during 10 min in acute post-stroke dysphagic patients showed a significant reduction in the pharyngeal transit time, swallowing response time and prevalence of aspirations, by increasing pharyngeal corticobulbar excitability and topographic representation in the undamaged hemisphere (Fraser et al. 2002). When this stimulation was carried out for three consecutive days (5 Hz, 10 min/day), it improved airway protection, reduced aspirations, improved feeding status and shortened time to decannulation and hospital discharge

after the intervention (Jayasekeran et al. 2010; Suntrup et al. 2015). A meta-analysis of three small clinical studies determined that intrapharyngeal electrical stimulation on stroke patients with OD reduced aspiration prevalence and hospital stay but demanded bigger studies to confirm these results (Scutt et al. 2015). A multicentre prospective single-blinded randomized controlled trial is currently being performed to validate previous findings (Dziewas et al. 2017).

- *Transcutaneous electrical stimulation*: Transcutaneous electrical stimulation is used to activate the muscles involved in swallowing function through the peripheral motor nerves (neuromuscular electrical stimulation, NMES) (Freed et al. 2001). However, their effectiveness and safety is still under discussion due to the inconsistent results (Logemann 2007; Ludlow et al. 2007; Oh et al. 2007; Rofes et al. 2013b). In addition to neuromuscular electrical stimulation, transcutaneous electrical stimulation has also been used as a sensory strategy, using lower electrical intensity to avoid muscle contraction during treatment. This approach has showed significant improvement in several swallow parameters, such as reduced swallow response time and prevalence of aspirations in chronic post-stroke dysphagic patients (Gallas et al. 2010; Rofes et al. 2013b) but not in Parkinson's disease patients with dysphagia (Baijens et al. 2013).

7 Future of Sensory Stimulation Treatment

This chapter has exposed the new generation of OD treatments based on the sensory stimulation of the OSR.

Older, stroke and neurodegenerative disease patients with OD present lower oropharyngeal sensitivity. This sensory loss is caused by either peripheral damage to the sensory innervation or impaired connectivity and activation within the CNS. Impaired oropharyngeal sensitivity is closely related to delayed OSR and weak tongue propulsion which cause the safety and efficacy impairments of deglutition in this population. That is why this new generation of treatments for OD are based in enhancing sensory perception.

These new sensory stimulation treatments are not only improving the OSR in OD patients in the short term but may have mid- and long-term effects on peripheral sensitivity and neurophysiology. As previously stated, long-term sensory stimulation of the oropharynx may promote the release of neuropeptides such as SP and CGRP that sensitize sensory receptors and thus improve peripheral sensitivity (Suntrup-Krueger et al. 2016; Nakato et al. 2017). Similarly, mid- and long-term sensory stimulation of the oropharynx has been shown to increase neuron excitability and cortical representation and activation, thus improving swallow response through neural plasticity mechanisms (Bonham et al. 2006; Ebihara et al. 2010). This neural plasticity could be related to the creation of new synaptic connections due to the long-term potentiation effects of sensory stimulation (Purves et al. 2012).

Despite its promising effects, not all OD patients may benefit from sensory stimulation treatments (Ortega et al. 2016; Nakato et al. 2017). The reason why some patients are non-responders to sensory stimulation can only be hypothesized. Sensory stimulation might be ineffective for patients with unaltered sensitivity and cortical activation or for patients with irreversible damage to afferent pathways and central sensory areas. Developing methods to predict responsiveness of patients to sensory stimulation treatment will be a milestone in the future treatment of OD patients.

In summary, the future of sensory stimulation treatment will be supported by two major pillars: on one hand, the research of new targets and the development of new sensory stimulation treatments; on the other hand, the development of non-invasive predictive tests of the responsiveness of patients to these therapeutic strategies.

Future treatment of older and neurological disease patients with OD will be personalized, with a comprehensive pathophysiological study that leads to the most suited therapeutic strategy for each patient. Future directions will take us

from current compensation of swallow disorders to the rehabilitation of the swallowing function with these future treatments.

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Chapter 31

Pharmacological use of transient receptor potential (TRP) ion channel agonists in neurological disease and aging: effects on swallowing and implications for nutrition

Noemí Tomsen[1,2] and Pere Clavé[1,2]

[1]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Instituto de Salud Carlos III, Barcelona, Spain;*
[2]*Gastrointestinal Motility Laboratory, Hospital de Mataró, Consorci Sanitari del Maresme, Mataró, Spain*

List of abbreviations

CGRP Calcitonin Gene-Related Peptide
CNS Central Nervous System
CPG Central Pattern Generator
EEG Electroencephalogram
ICD International Classification of Diseases
ISLN Internal Superior Laryngeal Nerve
LTSR Latency Time of the Swallow Response
LVC Laryngeal Vestibule Closure
OD Oropharyngeal Dysphagia
OSR Oropharyngeal Swallow Response
PAS Penetration-Aspiration Scale
PSEP Pharyngeal Sensory Evoked Potential
SP Substance P
TRP Transient Receptor Potential
TRPA1 Transient Receptor Potential Ankyrin 1
TRPC1 Transient Receptor Potential Canonical 1
TRPC6 Transient Receptor Potential Canonical 6
TRPM8 Transient Receptor Potential Melastatin 8
TRPV1 Transient Receptor Potential Vanilloid 1
TRPV2 Transient Receptor Potential Vanilloid 2
TRPV3 Transient Receptor Potential Vanilloid 3
TRPV4 Transient Receptor Potential Vanilloid 4
UESO Upper Esophageal Sphincter Opening

Mini-dictionary of terms

Calcitonin gene-related peptide: peptide member of the calcitonin family. It is associated with vasodilation and nociceptive processes and sensory input transmission.

Efficacy impairments refer to the presence of oral and/or pharyngeal residue after swallowing. It is related to complications such as malnutrition and dehydration.

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Oropharyngeal swallow response biomechanical swallowing response initiated with the change of the pharynx from a respiratory to a digestive configuration and finishing with the return to the respiratory reconfiguration. It includes, among other quantitative measures, the times to laryngeal vestibule closure and upper esophageal sphincter opening.

Penetration-aspiration scale eight-point scale where every score is determined by the depth of entry of a part of the food bolus to the airway and the ability to expel it.

Pharyngeal Sensory Evoked Potential measurement of cortical excitability in response to peripheral electrical stimuli in the pharynx.

Safety impairments refer to the entry of a part of the bolus to the airway, differentiating between penetrations (not passing the vocal chords) and aspirations (passing the vocal chords).

Substance P peptide member of the tachykinin family that can act as a neurotransmitter and as a neuromodulator. It is associated with pain, inflammatory processes, and sensory input transmission.

Time to laryngeal vestibule closure time from the glossopalatal junction opening to the closure of the laryngeal vestibule.

Time to upper esophageal sphincter opening time from the glossopalatal junction opening to the opening of the upper esophageal sphincter.

Videofluoroscopy Dynamic radiological evaluation of the deglutition process with a hydrosoluble contrast to assess signs of impaired efficacy and safety of swallow.

Introduction

Oropharyngeal dysphagia (OD) is a condition that consists of difficulty or discomfort during the progression of the alimentary bolus from the mouth to the esophagus. It is recognized by the World Health Organization with the International Classification of Diseases (ICD) codes 787.2 (ICD-9) and R13 (ICD-10). OD is very prevalent in several phenotypes of patient: 23%−51% in older people, 57%−84% in patients with neurodegenerative diseases, and 40%−81% in poststroke patients. However, it is underdiagnosed and undervalued in current clinical practice and does not have specific active treatment.

The main alterations caused by OD are classified in two groups: impaired efficacy (oral and pharyngeal residue) and safety (penetrations and aspirations). These alterations are associated with several health complications, worsening the quality of life of patients. Impaired efficacy is related to malnutrition and dehydration and is caused by impaired bolus propulsion force. Impaired safety causes respiratory infections, aspiration pneumonia, and an increase in the associated mortality and is related to increased time to laryngeal vestibule closure (LVC) and upper esophageal sphincter opening (UESO).

The impairment of the oropharyngeal sensory function is closely related to the alteration of the oropharyngeal swallow response (OSR) seen in older people and patients with neurologic or neurodegenerative disease and OD.

It is known that there is a correlation between oropharyngeal sensory threshold and age (Aviv, 1997), explained by the reduction of the number of small diameter, myelinated fibers in the internal superior laryngeal nerve observed in older people (Tiago, Pontes, & do Brasil, 2007). In addition, electroencephalographic studies have shown how older patients with and without dysphagia have increased latency and reduced amplitude of the characteristic peaks of the pharyngeal sensory potential (PSEP) due to the disrupted afferent input from the pharynx to the central nervous system (CNS). Therefore, pharyngeal sensory loss is closely related to increased prevalence of swallowing safety impairments (Rofes, Ortega, Vilardell, Mundet, & Clavé, 2017).

Oropharyngeal sensitivity can also be affected in stroke patients, increasing the prevalence of aspiration pneumonia due to the high risk of aspiration (Aviv, 1997). Note that cortical sensory representation is bilateral and symmetric, causing a reduction in oropharyngeal sensitivity independently of the hemisphere where the stroke occurs (Cabib et al., 2017). In contrast, the motor aspects of the swallow response can only be impaired if the stroke affects the dominant hemisphere for deglutition as oropharyngeal motor representation in the cortex is asymmetrical (Singh & Hamdy, 2006). Recent studies concluded that patients with poststroke dysphagia show a disrupted sensory input integration and reduced cortical excitability, in addition to the inability of the nonaffected hemisphere to recover the swallow motor response (Cabib et al., 2017, 2019).

Patients with neurodegenerative diseases, such as Parkinson's and Alzheimer's, also have impaired oropharyngeal sensitivity (Hammer, Murphy, & Abrams, 2013). This impairment is due to damage not only in the CNS but also in the peripheral nervous system, especially the sensory nerves that innervate the oropharynx (Mu et al., 2013). In addition, the presence of Lewy bodies has been described in the peripheral sensory nerve fibers of Parkinson's patients (Beach et al., 2010).

Oropharyngeal sensory inputs involve the afferent pathway of the neurophysiological response of swallowing, which transmits the stimulus from the oropharynx to the sensory cortex and the central pattern generator (CPG) and triggers the OSR through the efferent pathway. If the afferent pathway is impaired, as has been described in several phenotypes of patients with swallowing disorders, the efferent pathway may also be affected, resulting in impaired OSR (Cabib et al., 2016). The sensory pathway could therefore be a therapeutic target to improve the OSR, and the best way to treat the decrease in oropharyngeal sensitivity is through the transient receptor potential (TRP) channel agonists.

Transient receptor potential channel family

The presence of molecular receptors in the sensory nerves that innervate the oropharyngeal mucosa enables the perception of thermal and chemical stimuli. The TRP is the main family of sensory receptors found in the human oropharynx. Activation of these receptors, due to their capacity to perceive a wide spectrum of sensory stimuli, initiates the transmission of the sensory input from the oropharynx to several regions of the CNS (the sensory cortex and the dorsal swallowing group, DSG, located in the CPG) through the sensory nerves V, VII, IX, and X (Fig. 31.1).

TRPV1

The first TRP receptor identified was the subfamily vanilloid member 1 (TRPV1) (Caterina et al., 1997). The structure of TRPV1 ion channels is characterized by homotetramers, in which every subunit is formed by 6 transmembrane domains: two domains forming the pore of the channel and the other four performing the vanilloid binding site. The C-terminal and N-terminal of all transmembrane domains are located in the cytosolic space, allowing specific protein−protein interactions due to the presence of four to six ankyrin repeats. These protein−protein interactions are responsible for regulating channel function (Kedei et al., 2001; Mosavi, Minor, & Peng, 2002; Rosenbaum, Gordon-Shaag, Munari, & Gordon, 2004), principally the interaction with calmodulin, which regulates function according to intracellular calcium levels. Other important modulators are PIP2 (inhibiting channel activation) and PKA and PKC phosphatases (sensitizing the channel by the phosphorylation of their target amino acids) (Rosenbaum & Simon, 2007).

TRPV1 channels' main function is to detect harmful stimuli such as high temperatures (>43°C), acid solutions (pH < 5.5), and endogenous and exogenous chemical compounds such as capsaicin (Szallasi, Cortright, Blum, & Eid, 2007, Table 31.1). Activation of TRPV1 depends on the stimulus. High temperatures seem to affect several domains of the channel, while chemical agonists interact with the vanilloid binding site (Voets, 2014).

TRPV1 is mainly expressed in the afferent Aδ and C fibers and has been found in many human nerve (such as dorsal root ganglia and trigeminal ganglia) and nonnerve (epithelial cells) tissues. In addition, coexpression with some proinflammatory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) has been reported (Cortright et al., 2001; Denda et al., 2001; Ugawa, Ueda, Yamamura, Nagao, & Shimada, 2005).

In the oropharynx cavity, TRPV1 is found in the plasma membrane of the mucosa epithelial cells, in greater amounts in the superficial layers and decreasing through the intermediate layer cells to the basal lamina. In addition, it is found in the Aδ fibers innervating the mucosa and in some leukocytes located in the submucosa. TRPV1 is also found in the lingual mucosa, specifically on the membranes of the stratum basale and stratum spinosum cells of the filiform papillae epithelium and in the more superficial cell layers of the stratum granulosum and stratum corneum with a weaker signal expression (Figs. 31.2 and 31.3, Alvarez-Berdugo et al., 2016).

Activation with chemical and pharmacological compounds depends on the type of stimulus. Acidity in the oropharynx is one of the main factors that opens TRPV1 channels. When the pH is below 6.0, H^+ ions interact on the extracellular amino acids Glu-648, Val-538, and Thr-633, opening the pore. When the pH is between 6 and 7, the H^+ interacts with the amino acid Glu-600 and facilitates the activation of the channel by reducing the concentration threshold for other chemical agonists (Nilius, Owsianik, Voets, & Peters, 2007). Pungent compounds, such as capsaicin and other vanilloids, may increase the sensory input to the brainstem and cortical areas through the activation of the CPG and thus elicit the swallow response.

TRPV1 activation by the effect of its agonists stimulates the release of different neuropeptides, such as SP and CGRP, producing local effects through paracrine and autocrine mechanisms. The neuropeptide we currently have more information on is SP, which mediates the phosphorylation via PKC of the TRPV1 cytosolic domains, triggering the sensitization of the receptor and decreasing the activation threshold. In the larynx, SP can facilitate the motor swallow response through the sensitization of primary sensory neurons by direct action on sensory terminal nerves or the action of proinflammatory substances. SP also has a major role on the stimulation of the cough reflex (Nakagawa, Ohrui, Sekizawa, & Sasaki, 1995; Rofes, Cola, & Clave, 2014).

TRPA1

Following the description of TRPV1, the noxious cold stimuli receptor was identified: the transient receptor potential channel subfamily ankyrin member 1 (TRPA1), formerly called ANKTM1.

The TRPA1 channel is formed by homotetramers and each subunit has 6 transmembrane domains. One transmembrane domain of each subunit forms the pore of the channel. The most characteristic structure that distinguishes the TRPA1

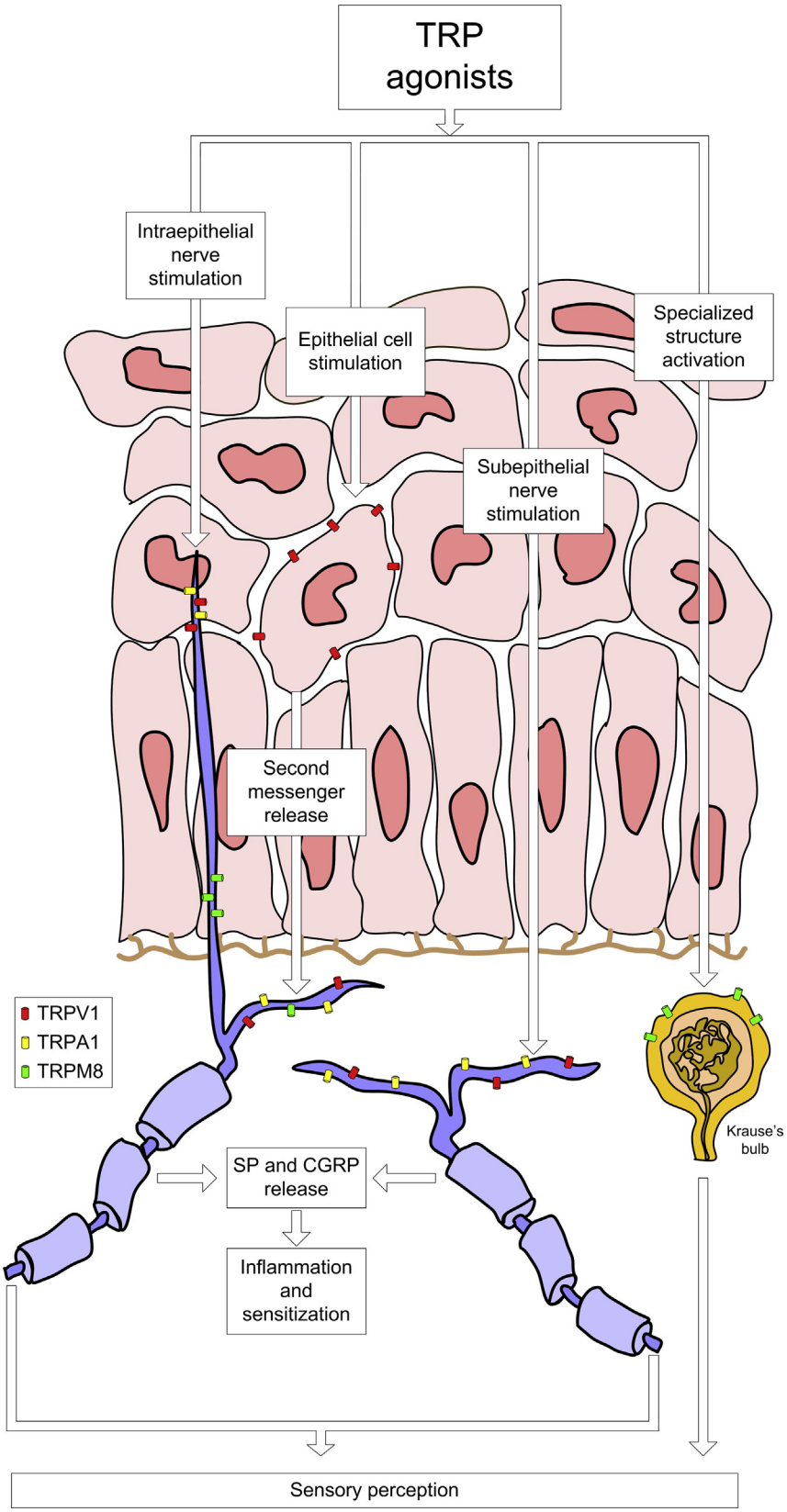

FIGURE 31.1 TRP agonist mechanism of action represented in the oropharyngeal mucosa. *CGRP*, Calcitonin Gene-Related Peptide; *SP*, Substance P; *TRPA1*, transient receptor potential subfamily ankyrin member 1 (yellow); *TRPM8*, transient receptor potential subfamily melastatin member 8 (green); *TRPV1*, transient receptor potential subfamily vanilloid member 1 (red).

TABLE 31.1 Main physical activators and natural agonists of the TRP receptor family.

TRPV1	TRPA1	TRPM8
Temperatures ≥43°C pH < 5.5	Temperature ≤17°C	Temperature 15−30°C
Exogenous chemical compounds: – Capsaicin – Allicin – Camphor – Cannabidiol and Cannabigerol – Eugenol – Gingerol – Piperine – Polygodial	Exogenous chemical compounds: – Piperine – 1,4-Dihydropyridines – Allicin – Allyl isothiocyanate – Cannabidiol, Cannabigerol, Cannabichromene, and Tetrahydrocannabinol – Capsiate – Cinnamaldehyde – Citral – Curcumin – Eugenol – Gingerol – Icilin	Exogenous chemical compounds: – Menthol – Eucalyptol – Geraniol – Icilin – Linalool
Endogenous chemical compounds: – Anandamide – Nitric oxide – Hydrogen sulfide	Endogenous chemical compounds: – Nitric oxide – Hydrogen sulfide – Bradykinin	

°*C*, Celsius degree; *TRPA1*, transient receptor potential subfamily A member 1; *TRPM8*, transient receptor potential subfamily M member 8; *TRPV1*, transient receptor potential subfamily V member 1.

channel from other TRP is the cytosolic N-terminal end, which has a spring shape due to its 17 ankyrin repeats (Sotomayor, Corey, & Schulten, 2005).

There are two kinds of stimuli that activate this receptor: noxious cold temperatures (<18°C) and natural or synthetic irritant substances (allyl isocyanate, cinnamaldehyde, piperine, allicin, allyl disulfide, and menthol, among others) (Bandell et al., 2004; Bautista et al., 2005; Karashima et al., 2007, Table 31.1). In addition, TRPA1 also has the ability to work as a mechanoreceptor in ear ciliate cells (García-Añoveros & Duggan, 2007).

TRPA1 colocalizes with TRPV1, SP, and CGRP in a subpopulation of C-fiber sensory neurons from the nodose, trigeminal, and dorsal root ganglia (DRG) (Kobayashi et al., 2005; Story et al., 2003). TRPA1 can also be found in fibroblasts and epithelial cells and bronchial walls (Atoyan, Shander, & Botchkareva, 2009; Mukhopadhyay et al., 2011).

Within the human oropharynx, TRPA1 can be found in the sensory nerve fibers below the basal lamina and the nerve fibers that penetrate the epithelium, the Langerhans cells of the stratum spinosum of the lingual mucosa, the lingual surface of the epiglottis mucosa, and the intermediate layer of the pharyngeal mucosa. In addition, TRPA1 is located in the sensory fibers that innervate the blood vessels that irrigate the submucosa (Figs. 31.2 and 31.3, Alvarez-Berdugo et al., 2016).

TRPM8

Another receptor related to thermal and chemical perception is the transient receptor potential subfamily melastatin member 8 (TRPM8). Its structure is a homotetramer composed of six transmembrane domains. Two of them form the pore of the channel while the other four act as the binding site of the menthol, as well as other agonists, and as a voltage sensor. The cytosolic domain is composed of the N terminal and C terminal domains and its extensive interfacial interactions are the most distinguished characteristic of the TRPM8 channel (Yin et al., 2017).

The activation of TRPM8 depends on nonharmful cold stimuli (15−30°C) and refreshing chemical substances such as menthol and eucalyptol (Table 31.1).

Unlike TRPA1, TRM8 is found in a subpopulation of small-diameter peripheral sensory nerves without coexpression with TRPV1 or CGRP (Peier et al., 2002). Regarding the oropharynx, TRPM8 is mainly expressed in the nerve fibers innervating the surface mucosa of the tongue, the oropharynx, and the lingual face of the epiglottis. These fibers, either in bundles or individually, are generally located below the epithelium but can cross the basal lamina and penetrate the deeper layers of the epithelium. It can also be found in the nerve fibers innervating the blood vessels that irrigate the submucosa (Fig. 31.2, Alvarez-Berdugo et al., 2018).

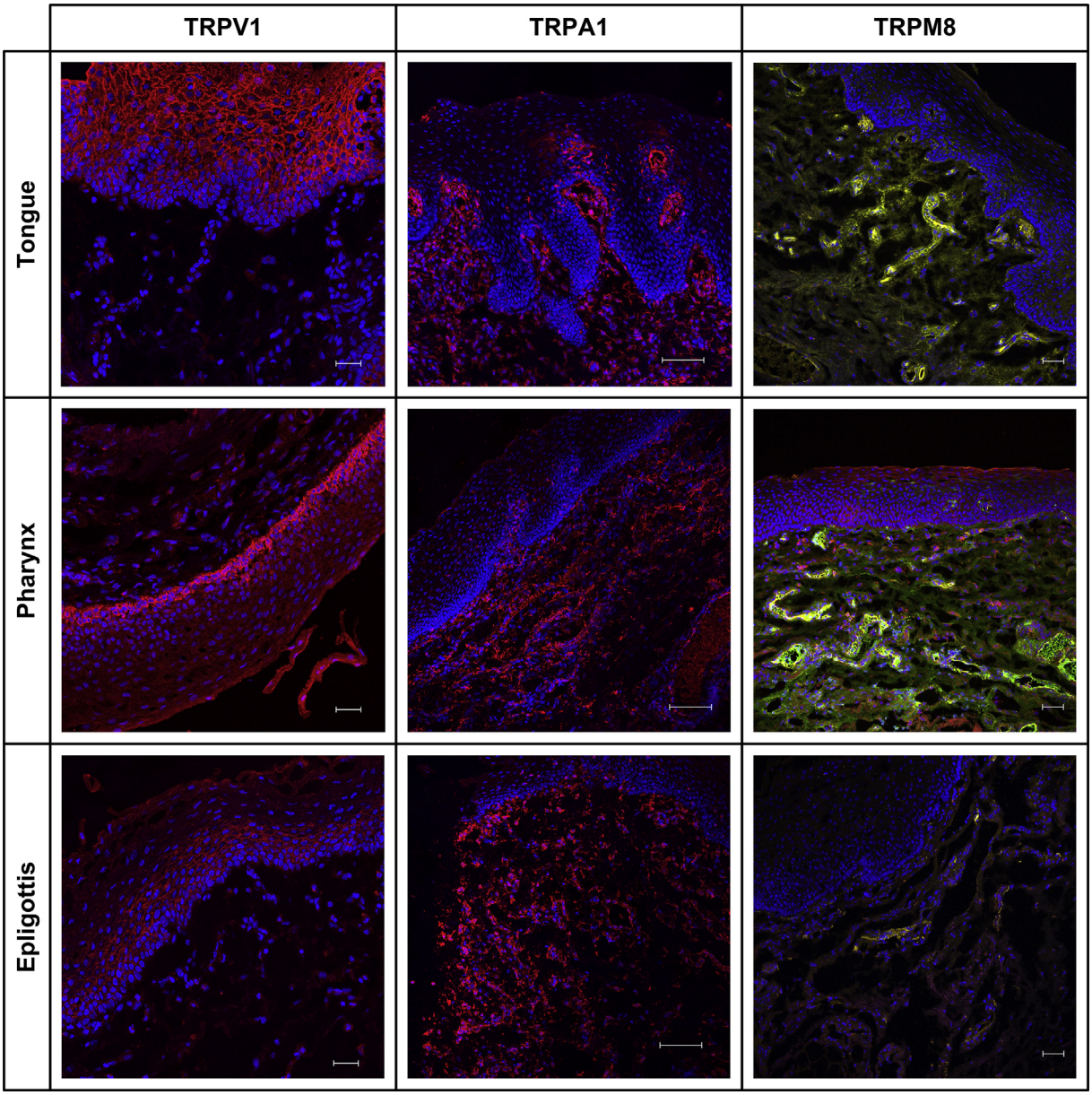

FIGURE 31.2 **Immunohistochemical images of TRP receptor localization in the human tongue, pharynx, and epiglottis.** *TRPA1*, transient receptor potential subfamily A member 1 (red); *TRPM8*, transient receptor potential subfamily M member 8 (green); *TRPV1*, transient receptor potential subfamily vanilloid member 1 (red). The cell nuclei are marked with DAPI (blue).

Other TRP

There are other TRP receptors that are involved in sensory perception apart from the channels TRPV1, TRPA1, and TRPM8, but little is known about their presence in the human oropharynx. Some of them are TRPV2, activated by temperatures above 52°C and found in rat pharyngeal mucosa, and TRPV3, which perceive temperature changes from 22 to 40°C (physiological range) and are located in mouse keratinocytes and neurons; TRPV4, which perceives osmotic changes and is found in mouse trachea and salivary glands; and TRPC1 and TRPC6, activated by harmful mechanical stimuli and osmotic changes (Alessandri-Haber, Dina, Chen, & Levine, 2009; Caterina, Rosen, Tominaga, Rosen, Levine & Julius, 1999; Chung, Lee, Mizuno, Suzuki, & Caterina, 2004; Delany et al., 2001; Peier, 2002; Sasaki et al., 2013; Xu, Ramsey, & Kotecha, 2002; Xu, Delling, Jun, & Clapham, 2006).

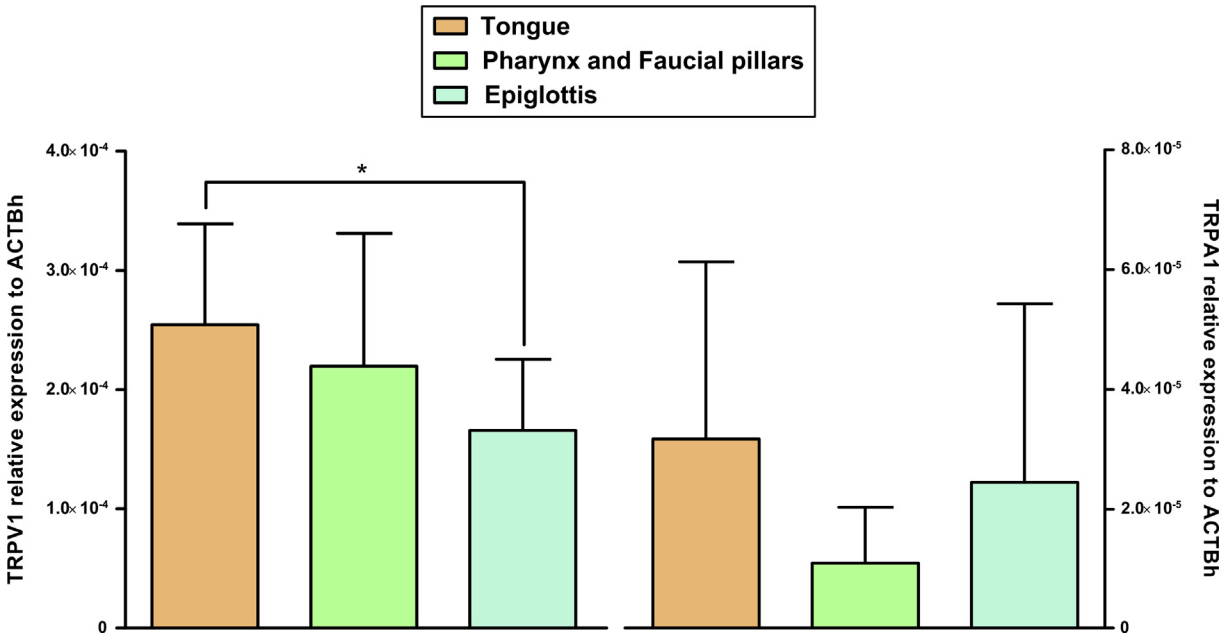

FIGURE 31.3 **Normalized expression of TRPV1 and TRPA1 in the human tongue, pharynx and faucial pillars, and epiglottis.** *ACTBh*, β-actin; *TRPA1*, transient receptor potential subfamily ankyrin member 1; *TRPV1*, transient receptor potential subfamily V member 1.

Chemical and pharmacological sensory stimulation treatments

Capsaicin

The first TRP agonist used was capsaicin, a pungent substance found in red chili peppers and the most studied TRPV1 agonist. Ebihara, Sekizawa, Nakazawa, and Sasaki (1993) observed a reduction in swallow reflex latency in a dose-dependent manner when they instilled capsaicin drops directly into the pharynx of stroke and dementia patients. Some years later, several studies have reported the efficacy of this agonist as an active treatment in two administration strategies in older patients with OD: acute (single dose) and subacute (multiple doses). Ebihara et al. (2005), again observed a reduction in the latency time of the swallow response (LTSR) and an improvement in the cough reflex in patients taking capsaicin troches. Our group also described that a single dose of capsaicinoids (150 μM) added to the alimentary bolus caused a reduction in the prevalence of both safety and efficacy impaired videofluoroscopic signs, in addition to reducing the time to LVC and to UESO and increasing hyoid and laryngeal displacement in patients with OD caused by age, stroke, or neurodegenerative diseases (Rofes, Arreola, Martin, & Clave, 2014a). Whereas, when the bolus was supplemented with a lower dose (10^{-5} M), the improvements in the biomechanics of swallowing were lost, and there were no effects on the neurophysiology. However, the multiple dose strategy with the lower dose showed better results. Taking 10 mL of a capsaicin solution three times a day for 10 days improved the penetration-aspiration scale (PAS) score, reduced the time to LVC by a 100 ms. In addition, a faster and more intense neurophysiological response and significant changes in brain activation were recorded. There was a positive correlation between the improvement of the conductivity of the stimuli from the pharynx to the sensory cortex and the shortening of the time to LVC (Tomsen et al., 2019). In another study, Nakato et al. describe a multiple-dose treatment where older patients with OD took capsaicin plus (0.75 μg of capsaicin) for 7 days and showed a reduction in the duration of the esophagus cervical wall opening and an increase in SP levels, as well as a correlation between these two improvements (Nakato et al., 2017).

Piperine

The second most studied TRP agonist is piperine, found in black pepper and a natural TRPA1 and TRPV1 agonist. One minute of olfactory stimulation with black pepper oil for 1 month in older patients with OD showed a shortening in the LTSR, as well as an increase in the serum SP levels and an improvement in swallowing frequency. In addition, as it is a direct stimulant of the insular cortex, it caused an increase in the regional cerebral blood flow (Ebihara, Ebihara, Maruyama, et al., 2006; Ebihara, Kohzuki, Sumi, & Ebihara, 2011). Another treatment approach is to supplement the

alimentary bolus with a high (150 μM) or low (1 mM) dose of piperine. After a single administration of the supplemented food bolus, patients with OD as a consequence of aging, neurological or neurodegenerative disease showed a reduction in the prevalence of nonsafe swallows and an improvement in the time to LVC. In addition, those patients that took the lowest dose also reduced their PAS score by about two points (Rofes, Arreola, Martin, & Clavé, 2014b).

Menthol

Menthol is one of the refreshing substances that act as a TRPM8 agonist and is found naturally in some aromatic plants such as mint. Despite scarce information on its role as an active treatment for swallowing disorders, the injection of 1 mL menthol solution (from 10^{-4} to 10^{-2} M) into the pharynx of older patients with OD is known to reduce LTSR in a dose-dependent manner (Ebihara, Ebihara, Watando, et al., 2006). In addition, we found that supplementation of the alimentary bolus with menthol at 10^{-2} M improved the swallow response in patients that had OD due to aging, stroke, or neurodegenerative diseases by reducing the time to LVC (Alvarez-Berdugo et al., 2017).

Combination treatment and comparative effect

Ebihara et al. described a treatment strategy in which three TRP agonists were introduced in a step-by-step manner: 3 days of olfactory stimulation with black pepper oil, 5 days of capsaicin troches, and finally, a menthol flavored jelly prior to oral feeding. This intensive stepwise treatment method began once the patients with OD had recovered from aspiration pneumonia and before oral food intake was reintroduced. They observed a decrease in the prevalence of pneumonia 1 month after the start of oral intake and a reduction in the number of patients that would need enteral tube feeding compared with the control group (Ebihara et al., 2010).

When Alvarez-Berdugo et al. compared the acute effect of the different TRP agonists, they saw that capsaicinoids (150 μM) reduced the prevalence of penetrations by 50%, the time to LVC by 24.38%, and pharyngeal residue by 80% and was the most effective active treatment tested. Piperine (150 μM) reduced the prevalence of penetrations and the time to LVC by 56.38% and 25.55% respectively but had no effect on pharyngeal residue, while menthol only reduced the prevalence of penetrations by 37.5% at a concentration of 1 mM and time to LVC by 18.44% at 10 mM. Therefore, menthol is the least effective of these agonists (Alvarez-Berdugo et al., 2017).

The future of sensory stimulation treatment

Although we have evidence of the effectiveness of TRP agonists in the short and mid-term, more studies are needed to describe the long-term effect of these treatments. These studies will reveal whether chronic administration of these compounds leads to desensitization.

Another important direction is to discover a peripheral biomarker in saliva that facilitates both the diagnosis and the degree of treatment response. The most studied candidate is SP, a neuropeptide secreted when TRP receptors are activated. Currently, an increase in the concentration of these neuropeptides (SP and CGRP) in saliva has been demonstrated to be a good marker for response to neurostimulation treatments such as capsaicin (Nakato et al., 2017; van Oosterhout et al., 2015). In addition, there are several studies that show the possibility of using SP as a diagnostic element in addition to those used in normal clinical practice. These studies described a lower basal concentration of SP in the saliva of OD patients, as a consequence of aging or a neurodegenerative disease, compared to those who do not have OD (Miarons et al., 2018; Schröder et al., 2019).

Finally, the results explained in this chapter show a hopeful future to develop an active treatment for swallowing disorders that will allow us to move on from compensation treatments to recovery of the swallowing function.

Applications to other areas of aging

Besides application as a possible active treatment for swallowing disorders, the effectiveness of capsaicin in other areas of aging has been demonstrated. The best known application is in the treatment of peripheral neuropathic pain, where the application of 8% capsaicin patches provides faster pain relief and fewer adverse effects than the optimal dose of pregabalin (Cruccu et al., 2018; Haanpää et al., 2016). The efficacy of capsaicin patches has also been demonstrated in the treatment of chronic pruritus, common among older patients (Cao, Tey, & Yosipovitch, 2018). In addition, an in vitro antioxidant effect of capsaicin has been described through the modulation of the redox status of the erythrocytes (Kumar, Chand, Chandra, & Maurya, 2015).

The antipruritic effect was also described with topical therapies containing 1%−3% menthol, which produce a refreshing effect on the skin and a reduction of pruritus in older patients (Patel, Ishiuji, & Yosipovitch, 2007).

Finally, oral treatment with piperine showed an improvement in cognitive function, the inhibition of Ach esterase, and an antioxidant effect in an aging rat model (Elnaggar, Etman, Abdelmonsif, & Abdallah, 2015).

Key facts of oropharyngeal dysphagia

- Oropharyngeal dysphagia (OD) is a condition that consists of difficulty or discomfort during the progression of the alimentary bolus from the mouth to the esophagus.
- It is a condition recognized by the World Health Organization in the International Classification of Diseases (ICD) with codes 787.2 (ICD-9) and R13 (ICD-10).
- The main causes of OD are aging, stroke, neurodegenerative diseases, head and neck cancer, and anatomic alterations.
- OD is very prevalent: 23%−51% in older people, 57%−84% in patients with neurodegenerative diseases, and 40% −81% in poststroke patients.
- There is no pharmacological treatment for OD. Standard treatment is based on compensatory strategies such as fluid adaptation with thickeners or postural changes to modify biomechanical alterations but does not rehabilitate the swallowing function.

Summary points

- Older and neurologic patients with swallowing disorders have impaired oropharyngeal sensory and motor function.
- Alteration of the afferent pathway of swallow neurophysiology contributes to the dysfunction of the efferent pathway, triggering an impaired swallow motor response.
- TRP receptor channels are located in the sensory fibers of the human oropharynx. Pharmacological activation of these receptors might in turn activate the afferent pathway and increase sensory input to the cortex and brain stem.
- TRP agonists in patients with oropharyngeal dysphagia have been demonstrated to be efficacious active treatment for swallowing disorders, improving both the biomechanics and the neurophysiology of swallowing.
- The development of an active pharmacological treatment will enable the rehabilitation of the oropharyngeal swallow response, moving from compensation to the recovery of brain and swallow function.

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