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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

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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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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

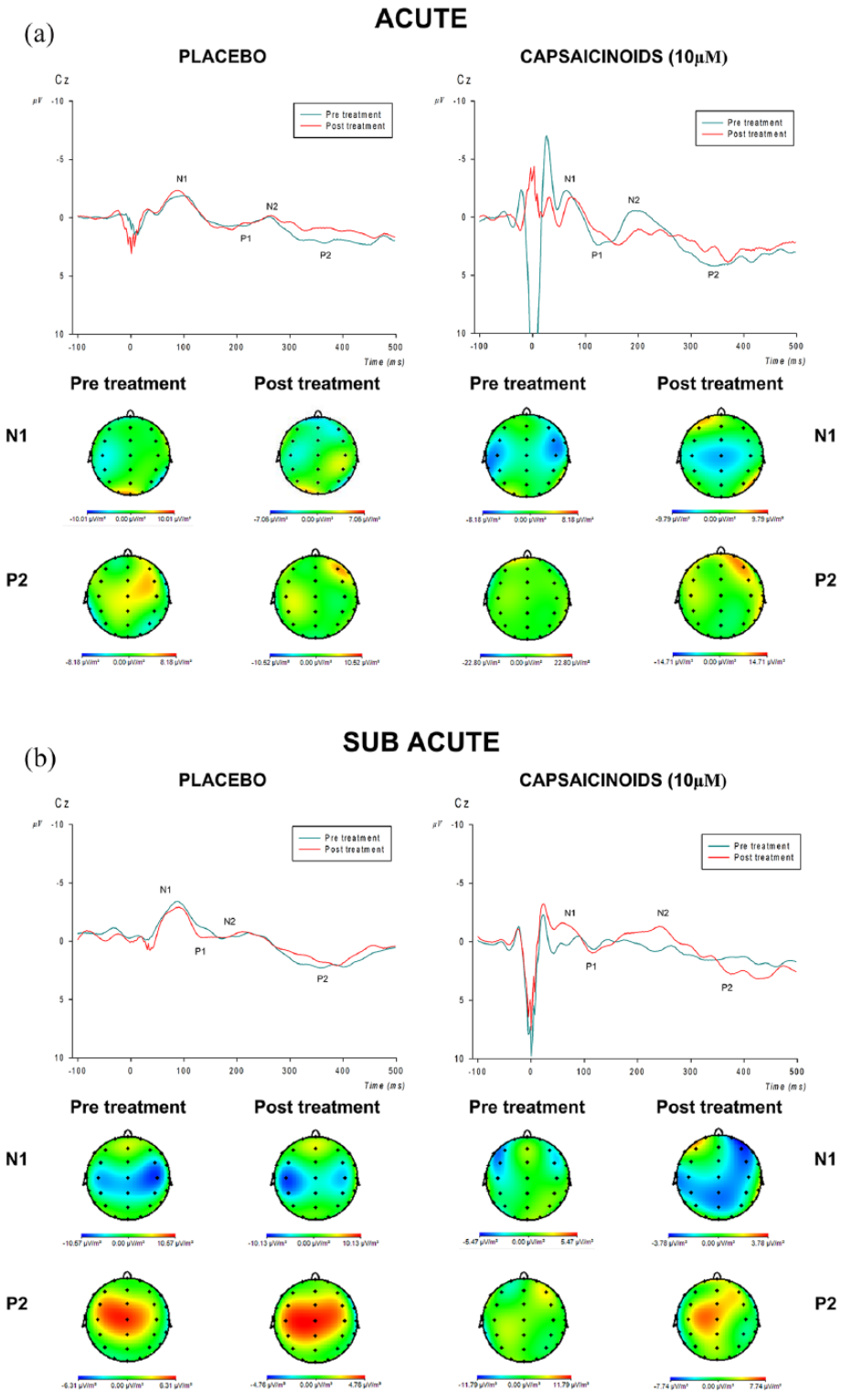

Figure 1. Pharyngeal event-related potential (ERP) and scalp density maps to pharyngeal ERPs for acute (a) and subacute (b) studies. At the top of each treatment, ERP traces obtained at the Cz electrode for pretreatment (blue line) and post-treatment (red line) from the placebo group and capsaicinoids 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

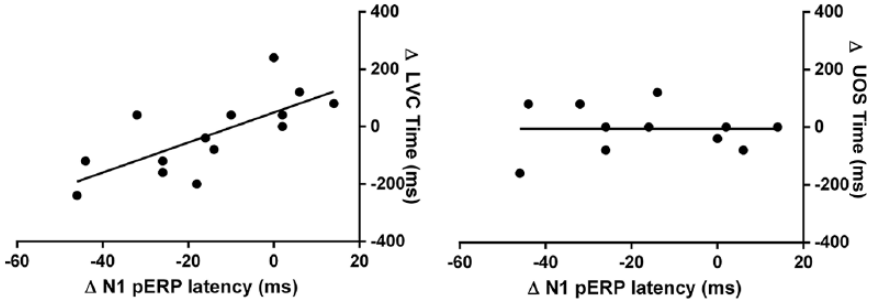

Figure 2. Correlation between the change in LVC time (left) and UOS closure time (right) and the reduction in the latency of N1 peak after the multiple-dose treatment.
LVC, laryngeal vestibule closure; pERP, pharyngeal event-related potential; UOS: upper oesophageal sphincter.

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

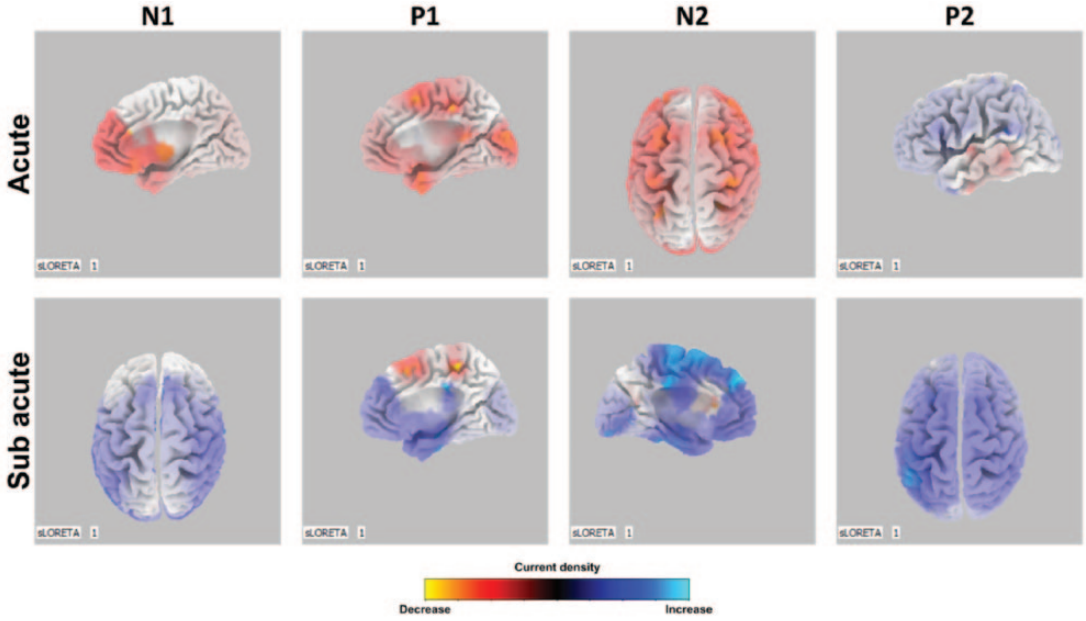

Figure 3. Differences in LORETA source activity after acute stimulation (top) and subacute stimulation (bottom) compared with basal cortical activity. Coloured voxels represent areas of significant differences in activation (blue, increase; red, decrease) after correction for multiple comparisons.

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.

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References

1. Clavé P and Shaker R. Dysphagia: current reality and scope of the problem. *Nat Rev Gastroenterol Hepatol* 2015; 12: 259–270.
2. Ekberg O, Hamdy S, Woisard V, *et al*. Social and psychological burden of dysphagia: its impact on diagnosis and treatment. *Dysphagia* 2002; 17: 139–146.
3. Baijens LWJ, Clavé P, Cras P, *et al*. European society for swallowing disorders – European Union Geriatric Medicine Society white paper: oropharyngeal dysphagia as a geriatric syndrome. *Clin Interv Aging* 2016; 11: 1403–1428.
4. Rofes L, Arreola V, Romea M, *et al*. Pathophysiology of oropharyngeal dysphagia in the frail elderly. *Neurogastroenterol Motil* 2010; 22: 1–9.
5. Ortega O, Rofes L, Martin A, *et al*. A comparative study between two sensory stimulation strategies after two weeks treatment on older patients with oropharyngeal dysphagia. *Dysphagia* 2016; 31: 706–716.
6. Logemann JA, Rademaker AW, Pauloski BR, *et al*. Normal swallowing physiology as viewed by videofluoroscopy and videoendoscopy. *Folia Phoniatr Logop* 1998; 50: 311–319.
7. Logemann J. *Evaluation and treatment of swallowing disorders*. Austin, TX: College-Hill Press, 1983.
8. Kahrilas PJ, Lin S, Rademaker AW, *et al*. Impaired deglutitive airway protection: a videofluoroscopic analysis of severity and mechanism. *Gastroenterology* 1997; 113: 1457–1464.
9. Robbins J, Langmore S, Hind JA, *et al*. Dysphagia research in the 21st century and beyond: proceedings from Dysphagia Experts Meeting, August 21, 2001. *J Rehabil Res Dev* 2002; 39: 543–548.
10. Logemann JA, Pauloski BR, Alfred W, *et al*. Temporal and biomechanical characteristics of oropharyngeal swallow in younger and old men. *J Speech Lang Hear Res* 2000; 43: 1264–1674.
11. Logemann JA, Pauloski BR, Alfred W, *et al*. Oropharyngeal swallow in younger and older women: videofluoroscopic analysis. *J Speech Lang Hear Res* 2002; 45: 434–445.
12. Robbins J, Humpal NS, Banaszynski K, *et al*. Age-related differences in pressures generated during isometric presses and swallows by healthy adults. *Dysphagia* 2016; 31: 90–96.
13. Hamdy S, Aziz Q, Rothwell JC, *et al*. The cortical topography of human swallowing musculature in health and disease. *Nat Med* 1996; 2: 1217–1224.
14. Hamdy S, Aziz Q, Rothwell JC, *et al*. Cranial nerve modulation of human cortical swallowing motor pathways. *Am J Physiol* 1997; 272: G802–G808.
15. Rofes L, Cola PC and Clave P. The effects of sensory stimulation on neurogenic oropharyngeal dysphagia. *J Gastroenterol Hepatol Res* 2014; 3: 1066–1072.
16. Cabib C, Ortega O, Vilardell N, *et al*. Chronic post-stroke oropharyngeal dysphagia is associated with impaired cortical activation to pharyngeal sensory inputs. *Eur J Neurol* 2017; 24: 1355–1362.
17. Rofes L, Ortega O, Vilardell N, *et al*. Spatiotemporal characteristics of the pharyngeal event-related potential in healthy subjects and older patients with oropharyngeal dysfunction. *Neurogastroenterol Motil* 2016; 29: 1–11.

18. Rofes L, Arreola V, Martin A, *et al*. Natural capsaicinoids improve swallow response in older patients with oropharyngeal dysphagia. *Gut* 2013; 62: 1280–1287.
19. Nakato R, Manabe N, Shimizu S, *et al*. Effects of capsaicin on older patients with oropharyngeal dysphagia: a double-blind, placebo-controlled, crossover study. *Digestion* 2017; 95: 210–220.
20. Ebihara T, Sekizawa K, Nakazawa H, *et al*. Capsaicin and swallow reflex. *Lancet* 1993; 341: 432.
21. Ebihara T, Takahashi H, Ebihara S, *et al*. Capsaicin troche for swallowing dysfunction in older people. *J Am Geriatr Soc* 2005; 53: 824–828.
22. Ebihara T, Ebihara S, Yamazaki M, *et al*. Intensive stepwise method for oral intake using a combination of transient receptor potential stimulation and olfactory stimulation inhibits the incidence of pneumonia in dysphagic older adults. *J Am Geriatr Soc* 2010; 58: 196–198.
23. Alvarez-Berdugo D, Rofes L, Arreola V, *et al*. A comparative study on the therapeutic effect of TRPV1, TRPA1, and TRPM8 agonists on swallowing dysfunction associated with aging and neurological diseases. *Neurogastroenterol Motil* 2018; 30: e13185.
24. Clavé P, Arreola V, Romea M, *et al*. Accuracy of the volume-viscosity swallow test for clinical screening of oropharyngeal dysphagia and aspiration. *Clin Nutr* 2008; 27: 806–815.
25. Mahoney FI and Barthel DW. Functional evaluation: the Barthel index. *Md State Med J* 1965; 14: 56–61.
26. Kaiser MJ, Bauer JM, Ramsch C, *et al*. Validation of the Mini Nutritional Assessment short-form (MNA-SF): a practical tool for identification of nutritional status. *J Nutr Health Aging* 2009; 13: 782–788.
27. Charlson ME, Pompei P, Ales KL, *et al*. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–383.
28. Baijens LW, Speyer R, Passos VL, *et al*. Swallowing in Parkinson patients versus healthy controls: reliability of measurements in videofluoroscopy. *Gastroenterol Res Pract* 2011; 2011: 380682.
29. Hind JA, Gensler G, Brandt DK, *et al*. Comparison of trained clinician ratings with expert ratings of aspiration on videofluoroscopic images from a randomized clinical trial. *Dysphagia*. 2009; 24: 211–217.
30. Kelly AM, Drinnan MJ and Leslie P. Assessing penetration and aspiration: how do videofluoroscopy and fiberoptic endoscopic evaluation of swallowing compare? *Laryngoscope* 2007; 117: 1723–1727.
31. Rosenbek JC, Robbins JA, Roecker PDEB, *et al*. A penetration-aspiration scale. *Dysphagia* 1996; 11: 93–98.
32. Klem GH, Lüders HO, Jasper HH, *et al*. The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl* 1999; 52: 3–6.
33. Ertekin C. Voluntary versus spontaneous swallowing in man. *Dysphagia* 2011; 26: 183–192.
34. Toogood JA, Smith RC, Stevens TK, *et al*. Swallowing preparation and execution: insights from a delayed-response functional magnetic resonance imaging (fMRI) study. *Dysphagia* 2017; 32: 526–541.
35. Alvarez-Berdugo D, Rofes L, Farré R, *et al*. Localization and expression of TRPV1 and TRPA1 in the human oropharynx and larynx. *Neurogastroenterol Motil* 2016; 28: 91–100.
36. Teismann IK, Suntrup S, Warnecke T, *et al*. Cortical swallowing processing in early subacute stroke. *BMC Neurol* 2011; 11: 34.
37. Suntrup-Krueger S, Bittner S, Recker S, *et al*. Electrical pharyngeal stimulation increases substance P level in saliva. *Neurogastroenterol Motil* 2016; 28: 855–860.