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Effects of olfactory and gustatory stimuli on neural excitability for swallowing

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ABSTRACT

This project evaluated the effects of olfactory and gustatory stimuli on the amplitude and latency of motor-evoked potentials (MEPs) from the submental muscles when evoked by transcranial magnetic stimulation (TMS). Sixteen healthy volunteers (8 males; age range 19–43) participated in the study. Lemon concentrate at 100% and diluted in water to 25% were presented separately as odor and tastant stimuli. Tap water was used as control. 15 trials of TMS-evoked MEPs triggered by volitional contraction of the submental muscles and volitional swallowing were measured at baseline, during control condition, during stimulus presentation, and immediately, 30-, 60-, and 90-min poststimulation for each of the four stimulus presentations. Experiments were repeated using the combined odor and tastant concentrations that most influenced the MEP independently. Differences in MEP amplitude measured during swallowing were seen at 30-, 60-, and 90-min poststimulation for simultaneous olfactory and gustatory stimulation as opposed to no differences seen at any point for stimuli presented separately. This study has shown that combined odor and tastant stimulation (i.e., flavor) can increase MEP amplitude during swallowing and that this enhancement of MEP can persist for at least 90 min following stimulation. As increased MEP amplitude has been associated with improved swallowing performance, a follow-up study is underway to determine the biomechanical changes produced by altered MEPs to facilitate translation of these data to clinical dysphagia management.

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1. Introduction

The neural substrates controlling swallowing are divided into three components [1]: (a) the afferent system comprising the trigeminal, glossopharyngeal, and vagus cranial nerves; (b) the brainstem swallowing center, constituting a central pattern generator; and (c) the higher centers which modulate the swallowing response [partly through the efferent system]. The central pattern generator for swallowing consists of two main groups of neurons, the dorsal swallowing group containing the generator neurons and ventral swallowing group, which are also known as the switching neurons [2]. The dorsal swallowing group, with the nucleus tractus solitarius as a central component, accepts sensory information relevant to swallowing and then sends information to the ventral swallowing group, which includes the nucleus ambiguous. Motor output for swallowing is executed through this group [2].

The central pattern generator for swallowing in the brainstem can be modulated by inputs from the periphery and cortex [3]. This modulation might include olfactory (smell) and gustatory (taste) components of food that are under preparation for swallowing. Several studies have revealed a cortical role in initiating and regulating swallowing function [3–5]. The cortex receives inputs from afferent nerves, integrates these inputs with information stored in other cortical areas (such as the limbic system), and then sends that input to the central pattern generator to modify motor output that is optimal for the bolus that a person is preparing to swallow [6].

Fibers from the lateral precentral gyrus (motor strip) are known to project to the nucleus tractus solitarius and to the nucleus ambiguus [7]. These projections could play a role in swallowing, specifically during the voluntary, preparatory stage. Moreover, it has been reported that fibers from the cortex terminate in the pontine and medullary reticular formation [8], which may influence the muscles innervated by motoneurons from these areas. Thus, information from the cortex may excite or inhibit motoneurons in coordinating muscle movements during swallowing.

Prior research has shown that motor neurons can also be excited or inhibited by extrinsic sensory stimulation [9]. Electrical stimulation to the pharynx has been found to modify motor-evoked potentials (MEPs) from pharyngeal muscles and also found to modulate subsequent swallowing function [9]. Thus, we proposed that other forms of sensory stimuli, such as smell and taste, could produce a similar effect and may also influence swallowing. There are many published studies which have evaluated gustatory effects on swallowing biomechanics [10–19]

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but only two studies which have investigated olfactory effects [20,21]. Studies which have evaluated the underlying neural effects of olfactory and gustatory stimulation are even scarcer, with a single report documenting effects of gustatory input on neural transmission [22]. How olfaction and gustation affect swallowing neural substrates is an important clinical question given the current approach of utilizing sensory modulation of taste and smell for rehabilitation of patients with dysphagia [12,14,16,20,21].

Corticobulbar excitation of the muscles involved in swallowing can be evaluated by measuring the MEP in the submental muscles. This is a measure of neural excitability from the motor cortex to target muscles [23,24] in which single-pulse transcranial magnetic stimulation (TMS) is used to noninvasively evoke the motor potential. TMS does this by depolarizing neurons in the motor cortex which generates action potentials and, subsequently, an MEP in the muscle(s) represented by the stimulated region of the motor cortex. This evoked potential can then be recorded by electromyography (EMG). At low intensity, TMS can indirectly excite the neurons to fire [25] or induce current changes in the motor cortex [26]. Larger and earlier-onset MEPs can be recorded when a muscle is preactivated, as opposed to recording during a rest condition, as the neurons are in a more active state under this condition [26,27]. More importantly, preactivation of the muscle during elicitation of an MEP provides valuable information regarding the functional relevance of motor pathway activation.

Submental muscles, composed of the anterior belly of digastric, mylohyoid, and geniohyoid muscles, are involved in superior and anterior movements of the hyolaryngeal complex, an integral biomechanical component of bolus transfer and airway protection [28]. Treatment approaches such as the head lift [29] and Mendelsohn maneuver [28] frequently target the submental muscle group. Other researchers have also reported increased submental muscle activation when sour stimuli were presented [11,13,17].

This study aimed to investigate the effects of two concentrations of lemon odor and tastant on the excitability of the corticobulbar pathways controlling the submental muscles. We hypothesized that stimulation by either smell or taste would change the amplitude of the MEP recorded in this muscle group. Furthermore, we hypothesized that a higher concentration stimulus would produce greater MEP amplitude than a lower concentration stimulus as increased molecular concentration of the stimulus may excite more receptors, thus increasing neural excitation. It was also hypothesized that simultaneous presentation of odor and tastant would produce greater MEP amplitude compared to independent presentation of either stimulus as the convergence of flavor processing on the neural systems would increase excitation [30,31].

As increased neural excitability has been shown to increase muscle activation, elucidation of the neural effects of smell and taste may support development of rehabilitation approaches for swallowing impairment which involve presentation of sensory stimulation. This may offer significant opportunities, in particular, for patients in whom cognitive deficits inhibit participation in more behaviorally-focused rehabilitation programs.

2. Methods

A repeated-measures within-subject design was used to evaluate the effects of olfaction and gustation on the neural substrates underlying swallowing. MEP measures were taken during and after stimuli presentation and compared with baseline data.

2.1. Participants

Based on a priori power analysis using data from this lab [24], 16 participants (8 males, 19–43 years, mean age 25.5 years, SD 7.6) were recruited. An equal number of males and females were used, as the ability to identify odor was reportedly better in women than in men [32]. Young healthy adults were chosen as the laryngopharyngeal sensory threshold is increased in healthy adults greater than 60 years of age [33].

The participants were in good health with no previous history of neurological problems or dysphagia. They were nonsmokers for at least 1 year prior to the study and were not taking medication that could affect swallowing function. Subjects were asked to refrain from ingesting caffeine, alcohol, or spicy food during the 12 h prior to the study [18,19,34]. This was to ensure that no chemical residuals from food were present on the taste receptors, which might alter taste stimuli. All participants were informed of the procedures and written consent was obtained prior to the experiments. Ethical approval was obtained from the regional Health and Disability Ethics Committee.

2.2. Equipment

A Magstim 200 (Magstim Company Ltd, Whitland, Wales, UK) transcranial magnetic stimulator with a figure-of-eight coil was used to evoke MEPs in the submental muscle group. The novel approach to evoke MEPs during both volitional contraction and volitional swallowing [24], as opposed to earlier research in which the MEPs were evoked during the rest condition [22], was used in this study. Submental muscle contraction activated the transcranial magnetic stimulator for both conditions. Muscle contraction was detected with surface EMG (sEMG) using an amplifier (Dual Bio Amps, Model ML135, ADInstruments, Castle Hill, Australia) and a recording system (PowerLab 8/30, Model ML870, ADInstruments, Castle Hill, Australia) which were connected to a custom-built trigger system. A DeVilbiss PulmoMate® compressor/nebuliser (Model 4650I, Sunrise Medical, Pennsylvania) was used to present olfactory stimuli via nasal cannulas (Airlife™ Adult Cushion Nasal Cannula with 2.1-m Crush Resistant Supply Tube, Cardinal Health, McGaw Park, IL).

2.3. Stimuli

A pilot study was completed to identify lemon stimuli at high and low concentrations that were tolerated well, readily identifiable to participants as “lemon”, and subjectively reported to be substantially different in intensity. Visual analog scales were used for 7 participants to document subjective ratings of intensity, pleasantness, and tolerability after randomized presentations of stimuli. Six concentrations of lemon odor and tastant were selected from the same source (Country Gold lemon juice, Steric Trading Pty. Ltd., Villawood, NSW, Australia) with concentrations below 100% diluted in water. High (100%) and low (25%) concentrations were ultimately chosen for inclusion in the study. Both stimuli were readily perceived by all participants as lemon odor and tastant, and the low concentration stimulus was perceived as being substantially more pleasant than the high concentration stimulus. Both stimuli were tolerated well by all participants, with the 100% stimulus being less well tolerated than the 25% stimulus.

Participants were exposed to the nebulised odor stimulus through a nasal cannula inserted in both nares. They were asked to breathe as usual. Nebulised tap water was used as control. Olfactory stimuli were presented continuously for a minute, then paused for 15 s to avoid adaptation [35,36]. The stimulus was then presented again for another minute, and this was repeated until all MEPs were recorded (see Experimental procedures).

Filter paper (Genuine Whatman Filter Paper No. 5, W & R Balston, England) cut into 8-cm by 2-cm strips were used to present the gustatory stimuli [37]. Five cm strips of filter paper were soaked with either of the two gustatory stimuli (low or high concentration) and allowed to air dry. These were then placed on the surface of the tongue at midline with the 5 cm strip covering approximately two-thirds of the length of the tongue from the anterior tip. By using this

method, chemical molecules of the tastant were dissolved in saliva and activated taste receptors in the taste buds on the tongue surface. Injection or ingestion of a taste substance in a fluid carrier would add the additional sensory input of bolus size and viscosity which would confound comparisons between sensory conditions. Blanks (impregnated with tap water) were used as control. A fresh taste stimulus was replaced after three swallows to ensure that all participants had the appropriate gustatory stimulus when MEPs were recorded (see Experimental procedures).

2.4. Experimental procedures

Participants were seated comfortably in a chair. Areas under the chin and overlying the ramus of the mandible were cleaned with an alcohol swab. A pair of electrodes (BRS-50-K/12 Blue Sensor, Ambu A/S, Ballerup, Denmark) for measurement of sEMG from submental muscles was placed at midline between the posterior aspect of the mandibular spine and the superior palpable edge of the thyroid cartilage; interelectrode distance was 5 mm. A ground electrode was placed over the ramus of the mandible.

To investigate task specific changes in MEP amplitude and latency, data were gathered during both volitional swallowing and volitional contraction tasks. These tasks were chosen to provide further information regarding the neural pathways engaged by pharyngeal swallowing. It is known that volitional contraction of the submental muscles, as in a stifled yawn, engages the corticobulbar pathway. It is less certain that pharyngeal swallowing, being a largely brainstem-driven task, utilizes this pathway, thus comparisons between these tasks may yield valuable information regarding swallowing neural control of swallowing.

Participants were first asked to practice the volitional swallowing and volitional contraction conditions that would trigger the TMS. For volitional swallowing, they were asked to swallow as they normally would but to minimize tongue movement. For the volitional contraction condition, the instruction was to “stifle a yawn” to attain contraction of the submental muscles. The participants were required to contract the muscles during both conditions to the approximate same amplitude, using sEMG output as a biofeedback modality to master motor performance.

The peak sEMG amplitudes of 10 swallows were averaged and 75% of this value was identified as the trigger threshold for that session. This threshold was consequently used to trigger TMS for both volitional contraction and volitional swallowing conditions. This was to ensure that the TMS was triggered at the same level of muscle activity in both conditions. The previously mentioned procedures were repeated at the beginning of each session.

The hotspot, or the location on the scalp that produces the most robust MEP in the submental muscles on stimulation, was then determined. Based on prior research, this was estimated to be approximately 4 cm anterior and 4 cm lateral to the vertex. Beginning from this point, the coil was moved in increments of 5 mm around the provisional spot while participants were asked to contract and briefly sustain contraction of the submental muscles. TMS was activated by the researcher at an intensity of 50% of the maximal TMS output. The intensity was increased in 10% increments, up to a level that was tolerated by the participant, if no MEPs were detected. This procedure was repeated until the hotspot was identified. This point was marked on the scalp and the same procedure was repeated in the opposite hemisphere.

After bilateral hotspots were identified, a stimulus response curve was derived to determine TMS intensity output that is appropriate for the participant. With the coil at one hotspot in either hemisphere, the area was stimulated three times, starting with a TMS intensity that produced no MEP response. The intensity was increased in 10% steps until the MEP reached maximal amplitude (i.e., did not increase in amplitude with higher TMS intensity). Three MEPs with maximal amplitude were then averaged. The TMS intensity that produced 50% of this amplitude was the intensity used for all sessions. These procedures were repeated in the other hemisphere to determine the dominant hemisphere, which is the hemisphere that produces a more robust MEP with the lowest TMS intensity. Subsequent trials were carried out only on the dominant hemisphere.

Fifteen MEPs during volitional swallowing and 15 MEPs during volitional contraction were recorded at baseline, during the control condition, during stimulus presentation, immediately poststimulation, and at 30-, 60-, and 90-min poststimulation in four separate sessions. The EMG-activated triggering system was locked for a period of 10 s after each contraction/swallow to avoid accidental triggering. Water to moisten the oral mucosa was regularly offered between contractions/swallows. The swallowing and contraction conditions were counter-balanced across sessions. Each session included exposure to stimulation by low odor, high odor, low tastant, or high tastant stimuli in random order across participants. Water was used during the control condition. After analysis of preliminary data, the odor and tastant that maximally influenced the MEP in each participant, irrespective of excitatory or inhibitory response, were then presented simultaneously in another session.

2.5. Data analyses

As MEP responses can vary considerably between subjects [38], analyses were based on percent change in amplitude or latency from baseline. Data were analyzed using SPSS 17 (SPSS Inc.). Repeated-measures ANOVA were performed to evaluate the effect of concentration and time on both odor and tastant during volitional contraction and volitional swallowing. Tests on concentration were performed separately at two levels (low and high), and then collapsed as “odor” or “tastant” if there were no differences in MEP amplitude or latency as a function of concentration. Further ANOVAs were then performed on odor and combined stimulation, or on tastant and combined stimulation. For all analyses, combined stimulation refers to the simultaneous presentation of odor and tastant. Sex was selected as a covariate in all analyses initially, and analyses were re-run without sex if it was not significant.

The immediate effect of stimulus was evaluated by comparing MEPs between control condition and during stimulation. The effect of stimulus across time, or late effect, was assessed by comparing the MEPs at baseline with MEPs immediately poststimulation (5 min) and at 30-, 60-, and 90-min poststimulation. Posthoc *t*-tests were performed where ANOVAs were significant. $p<0.05$ was taken as significant. For all repeated-measures analyses, Greenhouse–Geisser correction was reported if the assumption of sphericity was violated (when Mauchly's test of sphericity was significant).

3. Results

MEPs for volitional contraction were recorded from all 16 participants but only 9 participants had recordable MEPs during volitional swallowing.

3.1. Volitional contraction

3.1.1. MEP amplitude during volitional contraction: Immediate effect

No differences in MEP amplitude were detected between low and high concentrations of odor. Further analyses between odor and combined stimulation were also nonsignificant. The percent changes from baseline in MEP amplitude were greater for the high than for the low concentration tastant [20.1 ± 40.3 vs 0.1 ± 25.6; $F(1, 15) = 4.7$, $p = 0.048$]. Therefore, low and high tastants were not collapsed in this analysis. Results of ANOVAs on MEP amplitude for both tastant stimuli and combined stimulation were also nonsignificant.

3.1.2. MEP amplitude during volitional contraction: Late effect

No differences in MEP amplitude were detected between low and high concentrations of odor or tastant; therefore, the two concentrations of each stimulus were collapsed to represent a single stimulus for odor and another for tastant. There was no main effect of stimulus (odor vs combined stimulation and tastant vs combined stimulation) or time (5-, 30-, 60-, and 90-min poststimulation) on MEP amplitude for odor, tastant, or combined stimulation. However, there was an interaction between stimuli (odor and combined stimulation) and time [$F(2.3, 34.4) = 4.7$, $p = 0.013$]. Posthoc *t*-tests revealed differences in MEP amplitude for odor at 90 min poststimulation compared to baseline [573 ± 327 μV vs 502 ± 297 μV; $t(15) = 2.2$, $p = 0.046$].

3.1.3. MEP latency during volitional contraction: Immediate effect

No differences in MEP latency were detected between low and high concentrations of odor or tastant when they were compared with their respective control conditions. Further analyses on odor, tastant, and combined stimulation during control condition and during stimulation showed no significant main or interaction effect on MEP latency (raw data shown in Table 1).

3.1.4. MEP latency during volitional contraction: Late effect

No main effect of concentration (low and high) was identified for MEP latency of either stimulus; therefore, they were collapsed for single concentrations of odor and of tastant. There was no main effect of stimulus (odor vs combined stimulation and tastant vs combined stimulation), time (5-, 30-, 60-, and 90-min poststimulation), or interaction effect between stimuli and time on MEP latency for odor, tastant, or combined stimulation (raw data shown in Table 2).

3.2. Volitional swallowing

3.2.1. MEP amplitude during volitional swallowing: Immediate effect

No main effect of concentration (low and high) in MEP amplitude was detected for odor or tastant, so they were collapsed as odor and tastant. Further analyses on odor, tastant, combined stimulation, and their control conditions showed no significant main effect of stimulus on MEP amplitude (raw data shown in Table 3).

3.2.2. MEP amplitude during volitional swallowing: Late effect

No differences in MEP amplitude were detected between low and high concentrations of odor or tastant; therefore, they were collapsed as odor and tastant. There was a main effect of time on MEP amplitude during swallowing when two-way ANOVA was run for odor and combined stimulation [$F(4, 32) = 2.8$, $p = 0.042$].

Sex was significant in the interaction between tastant and combined stimulation [$F(4, 28) = 3.7$, $p = 0.015$]. The interaction effect was also significant [$F(4, 28) = 4.8$, $p = 0.004$]. Posthoc *t*-tests were significant for MEP amplitude during volitional swallowing at 30-, 60-, and 90-min poststimulation for combined stimulation only [$t(8) = 2.7$, $p = 0.026$; $t(8) = 2.4$, $p = 0.046$; and $t(8) = 2.9$, $p = 0.019$; respectively] (Figs. 1 and 2).

3.2.3. MEP latency during volitional swallowing: Immediate effect

No differences in MEP latency were detected between low and high concentrations of odor or tastant; therefore, they were collapsed as odor and tastant. Further analyses on tastant and combined stimulation showed an effect of stimulus for MEP latency [$F(1, 8) = 5.7$, $p = 0.045$] between control condition and during stimulation (Fig. 3).

Table 1
Raw data for mean MEP latency (SD) during volitional contraction for immediate effect.

	Mean MEP latency (ms) (SD)		
	Odor stimulation	Tastant stimulation	Combined stimulation
Control condition	9.8 (1.0)	9.4 (0.7)	9.3 (0.8)
During stimulation	9.3 (0.8)	9.2 (0.9)	9.5 (0.9)

Table 2
Raw data for mean MEP latency (SD) during volitional contraction for late effect.

	Mean MEP latency (ms) (SD)		
	Odor stimulation	Tastant stimulation	Combined stimulation
Baseline	9.4 (0.9)	9.2 (0.8)	9.5 (0.8)
5 min post	9.4 (0.7)	9.2 (0.8)	9.3 (0.8)
30 min post	9.5 (0.8)	9.2 (0.9)	9.3 (0.9)
60 min post	9.3 (0.8)	9.2 (0.9)	9.4 (1.0)
90 min post	9.2 (0.8)	9.2 (0.9)	9.5 (0.9)

3.2.4. MEP latency during volitional swallowing: Late effect

No differences in MEP latency were detected between low and high concentrations of odor or tastant. Further analyses on odor, tastant, and combined stimulation showed no effect of stimulus or time on MEP latency, and no interaction effect (raw data shown in Table 4).

4. Discussion

Our study is the first to demonstrate changes in MEP amplitude during volitional swallowing following simultaneous presentation of odor and tastant stimuli. It has also shown that these increases in MEP amplitude were not present immediately poststimulation but were evident from at least 30- to 90-min poststimulation. Additionally, odor presentation was found to influence the excitability of the neural pathway during volitional contraction but the effect was only evident 90 min poststimulation. No long-term effects were found when tastant was presented independently. As our odor presentation was nebulized via nasal cannula inserted into both nares, odor molecules may also have stimulated some taste buds in the nasopharynx. Tastant stimulation alone did not stimulate the odor receptors as the filter paper was placed anteriorly on the tongue surface, which may not stimulate the retronasal odor receptors.

4.1. Motor-evoked potentials (MEP)

MEPs are a measure of neural excitation from the motor cortex to the target muscles [23,24]. This study evaluated MEPs when the submental muscles were partially contracting for two reasons. First, it is known that MEPs are larger when recorded during preactivation [26,27] and prior research on MEPs associated with muscles of the head and neck has shown that MEPs can best be elicited when background muscle contraction is present [39,40]. A custom-built trigger system was used to monitor muscle contraction and ensure that the TMS output was triggered at the same level of muscle contraction for both tasks to avoid a systematic measurement error. More importantly, we wanted to evaluate the cortical contribution during brainstem-controlled swallowing activity and compare this to a less complex and better defined pyramidal motor task of the corticobulbar pathway during volitional contraction of the submental

Table 3
Raw data for mean MEP amplitude (SD) during volitional swallowing for immediate effect.

	Mean MEP amplitude (μV) (SD)		
	Odor stimulation	Tastant stimulation	Combined stimulation
Control condition	419.8 (178.3)	450.5 (107.0)	496.5 (189.1)
During stimulation	405.1 (159.4)	442.4 (136.1)	464.7 (148.8)

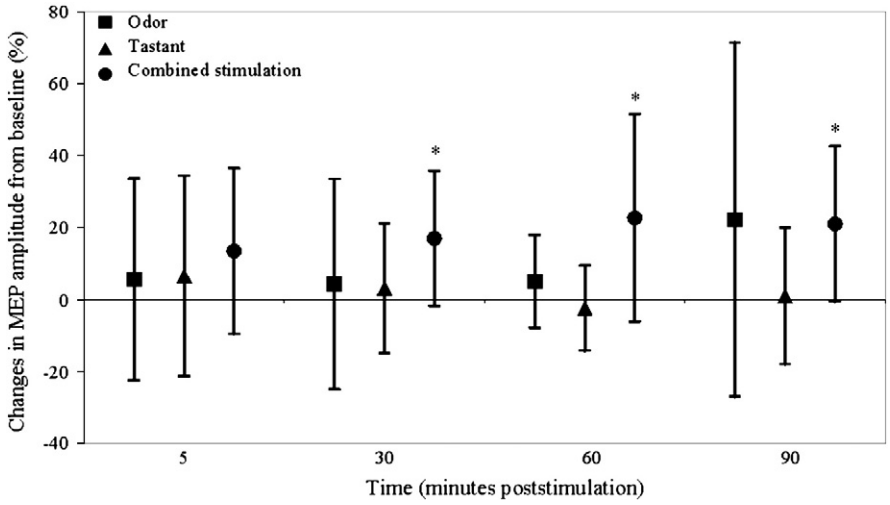

Fig. 1. Percent changes from baseline (mean and SD) in MEP amplitudes for odor, tastant, and combined stimulation during volitional swallowing immediately poststimulation (5 min), at 30-, 60-, and 90-min poststimulation; $^{*}p<0.05$.

muscles. Our research results justify this approach as there were notable differences in task-related MEPs.

At baseline, MEPs recorded during volitional contraction were recorded from all 16 participants but only 9 participants had recordable MEPs during volitional swallowing. This finding is consistent with prior research from our lab [41], which found MEPs to be more robust during volitional contraction than during swallowing. It has been hypothesized that this may be due to greater cortical drive utilization during the contraction condition, compared to the brainstem-activated swallowing condition which uses less cortical input [41]. If the corticobulbar pathway is not substantially pre-activated during swallowing, the MEP output from TMS is not boosted, resulting in very small or immeasurable MEPs at the periphery [39]. Another interpretation is that the primary motor cortex exerts an inhibitory influence on swallowing neural networks, thereby minimizing the measured MEP output from the excitatory TMS input [23].

Increases in MEP amplitude were significant during swallowing following simultaneous odor and tastant stimulation, suggesting that the presentation of a single modality is insufficient to evoke changes in the MEP during swallowing. However, independently presenting odor stimulus is enough to change the MEP during volitional contraction, albeit with a more prolonged delay in the change (90 min for contraction compared to 30 min for swallowing). As no MEP changes were seen with tastant presentation, and odor can stimulate taste buds in the nasopharynx, we proposed that single sensory modality is also not enough to change the MEP during volitional contraction. The simultaneous presentation of odor and tastant is flavor, which is considered to represent a separate sensory stimulus, rather than merely a combination of the independent stimuli of smell and taste [30,42].

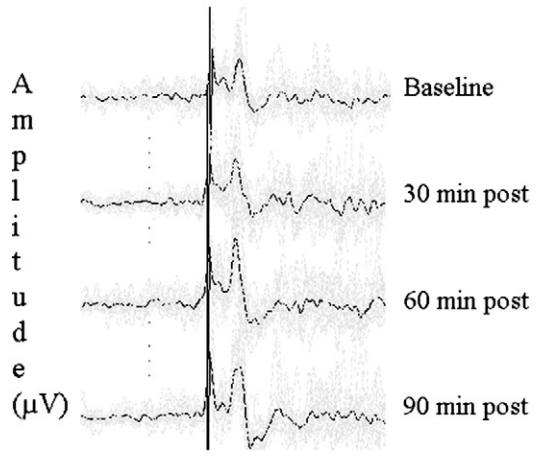

Fig. 2. MEP waveforms of one participant during volitional swallowing at baseline and at 30-, 60-, and 90-min following simultaneous odor and tastant presentation, 15 MEPs are superimposed with the average MEP in bold.

4.2. Cortical regions involved in swallowing

The olfactory and gustatory pathways converge onto neurons in the endopiriform nucleus. Human interest in food is modulated by mechanisms related to the cortical integration of olfactory and gustatory information in this nucleus, which is located between the piriform cortex and caudate–putamen [43]. The insula has also been implicated as an area where smell and taste information are integrated. Lesions in the anterior insula are related to dysphagia but not when confined to the posterior insula [44], suggesting that the anterior insula is an important area in regulating swallowing function. Furthermore, gustatory aura has been noted to precede epileptic convulsions in people with injury to the anterior insula [45]. It has been shown that information from the anterior insular travels to the nucleus tractus solitarius (NTS) in the brainstem [46]. Activities in the NTS have been suggested to modulate the brainstem interneurons, hence the muscles involved in swallowing [22]. Additionally, the NTS may receive increased information from other brain areas activated by flavor stimulation. Signals from the piriform cortex travel via the mediodorsal nucleus of the thalamus to the prefrontal cortex [47], and, in turn, to the supplementary motor area [48]. Information from the supplementary motor area can be directly channeled to the brainstem [49]. Furthermore, the reticular area in the brainstem also receives information from the cortex [8]. Specifically for swallowing, it has been suggested that information from the cortex can modulate interneuronal activities in the dorsal swallowing group (NTS), which can then change neuronal activities in the nucleus ambiguus [50]. Furthermore, it has been reported that information from the dorsal medullary area ventral to the NTS has connections to the trigeminal, facial, and hypoglossal motor nuclei [51]. Therefore, changes in brainstem neuronal activity can modify the contraction of muscles innervated by these motor nuclei.

Multiple cortical regions, as described earlier, may all contribute to adaptation in the NTS, probably through increased number of motoneurons activated. Thus, it can be speculated that the increased

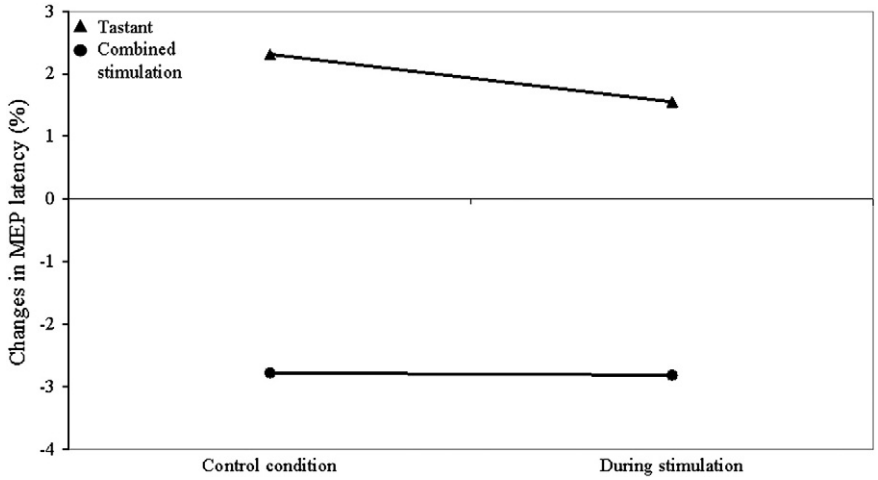

Fig. 3. Percent changes from baseline in MEP latencies for tastant and combined stimulation during swallowing for control condition and during stimulation.

MEP amplitude seen in this study during swallowing was due to increased activation in the NTS following simultaneous odor and tastant stimulation. In addition, the MEP latency for swallowing was also decreased during stimulation only when odor and tastant were presented simultaneously, indicating an excitatory effect of the combined stimulation on the speed of neural transmission.

4.3. Effects of sour taste on swallowing

Sour taste has been shown to have widely differing effects on swallowing biomechanics, which could reflect methodological differences between the studies. Some authors have reported better swallowing function when healthy participants or patients were given sour tastant [11,13,14,16,17], whereas another reported poorer swallowing behavior [10], and yet another reported no changes [12]. To our knowledge, no study has reported the effect of sour taste on neural transmission during swallowing. However, there is a study which evaluated corticobulbar excitability in healthy adult males following pleasant (sweet) and aversive (bitter) taste stimuli by measuring pharyngeal MEP triggered with TMS [22]. A delayed (30 min) inhibitory effect on swallowing for both stimuli, seen as decreases in pharyngeal MEP amplitude was reported. The conclusion from that study was that taste stimuli directly reduced activity in the NTS, which then caused a "reduction in the activity of cortical swallowing centers" [22]. The findings appear to be in opposition to the clinical use of sour bolus to facilitate more timely swallowing [14]. The authors attributed their results to a behavioral consequence of the strong flavor used.

In another study, glucose, citrus, and saline reportedly decreased the rate of ingested bolus per swallow during a water swallow test in normal adults; the outcome is similar to the effect of anesthesia [10]. The authors proposed that as most of the participants rated the tastants as intense, the "heightened sensory input" increased the participants' alertness as a protective mechanism towards noxious stimuli, thus the decreased rate of ingested bolus. The swallowing results seen in these studies [10,22] were thought to be due to the participants' perception of the stimuli as being noxious. Another explanation was that the stimulus has an effect on trigeminal stimulation, which is mediated by free nerve endings of trigeminal nerve axons in the olfactory mucosa and oral cavity [52], usually as a result of irritating chemicals. To ensure that we did not encounter this problem, a pilot study was conducted to identify a suitable stimulus. In the current study, two concentrations of each olfactory and gustatory stimulus were used: a higher concentration which was rated by participants as acceptable but not pleasant, and a lower concentration which was deemed acceptable and more pleasant. Sensation of taste is a "combination of gustatory and olfactory information" [53]; therefore, we wanted to use an odor that would resemble the sour tastant, hence the use of nebulised lemon odor from the same source.

In this study, sex was found to be a significant factor in the interaction between tastant and combined stimulation. Previous studies have mixed results on sex effects. However, a study on parotid saliva flow following taste stimulation reported increased flow rate in males compared to females [54]. Our study found that males have larger MEP amplitude compared to females when taste was presented. It would be interesting to further investigate how MEP is affected by bolus volume, considering that increased salivary flow would correspond to increased bolus volume.

Our finding that smell and taste enhanced swallowing neural control differs from previous results [10,22] which reported poorer swallowing function following taste stimulation. The differences may be explained by the different stimuli used and the fact that the MEPs were recorded from different sites under a different condition (at-rest vs voluntary contraction). Furthermore, we incorporated two different sensory stimuli, which are known to excite a different brain region than any of them given independently. Neurons in the NTS can be excited or inhibited by taste stimulus [3]; the stimulus used in the present study excited these neurons, in contrast to the stimuli used in the earlier studies. Our data seem to support the clinical suggestion that sour bolus facilitates swallowing.

We also sought to evaluate if any olfactory and gustatory effects were present after the stimuli were removed, thus the recordings of MEPs up to 90 min poststimulation. Decreased MEP amplitude following taste stimulation 30 min poststimulation has been previously reported [22], and data from this lab reported increased MEP amplitude in response to electrical stimulation 60 min poststimulation [55]. Late changes in the MEP amplitude may be explained by residual odor and tastant molecules that were present after the stimulus was taken away, allowing the receptors to be activated poststimulation. However, given the long latency of response, we

Table 4
Raw data for mean MEP latency (SD) during volitional swallowing for late effect.

	Mean MEP latency (ms) (SD)		
	Odor stimulation	Tastant stimulation	Combined stimulation
Baseline	9.2 (1.0)	9.5 (0.9)	9.4 (0.9)
5 min post	9.2 (0.7)	9.5 (1.1)	9.3 (1.2)
30 min post	9.4 (0.7)	9.1 (1.1)	9.1 (1.1)
60 min post	9.0 (0.6)	9.1 (1.1)	9.2 (1.4)
90 min post	8.9 (0.7)	9.2 (1.0)	9.1 (1.0)

propose that changes in the MEP amplitude at 30-, 60-, and 90-min poststimulation are more likely explained by way of long-term potentiation (LTP), which has been implicated as one aspect of neural plasticity [56]. LTP is an increase in synaptic strength transmission, which can be achieved with persistent stimulation of a synapse. Repetitive activation will lead to several mechanisms which would eventually change the physiology of the synaptic membrane for a more efficient transfer of neural signals by, for example, increasing the number of receptors in the membrane [56]. Moreover, studies in animal model showed that LTP was present long after the stimulation was removed, indicating auto-regulation of the sensory-motor network towards the initial stimulus [57]. This evidence of neural plasticity may thus contribute to long-term, rehabilitative recovery in patients with swallowing impairment.

Simultaneous stimulation of smell and taste could provide an optimal sensory condition for mimicking real food which would increase swallowing efficiency. We are currently looking at combined stimuli effects on swallowing biomechanics to further help us translate these data into dysphagia management. Investigating the effects of smell and taste on swallowing function in the elderly and in patients with dysphagia would further increase our knowledge on sensory manipulation in the treatment of dysphagia.

4.4. Limitations

There were some limitations in our study, which deserve discussion. Sour stimuli can increase salivation [58] and, in turn, the volume of ingested saliva and spontaneous swallowing. However, the increase in saliva flow following lemon juice stimulation is reported to be less than 0.3 ml/30 s [58]. Although bolus volume is known to affect swallowing function, anything less than 1 ml is considered too small to have any effect [14,59]. Spontaneous swallowing was not controlled for in the study but MEPs were only recorded when the system was activated by breaching the EMG threshold. By using the same threshold for both swallowing and contraction conditions, we can assume that the amount of muscles preactivated when TMS was triggered is the same.

Odors are able to evoke one's memory and emotion [35,53]. The amount of odor molecules stimulating a person's olfactory neurons depends on the concentration of the stimulus. A person has to sniff to improve olfaction, as less than 10% of the air we breathe in reaches the olfactory epithelium [60], but participants were given instructions to breathe normally through their nose during all procedures to ensure that the amount of odor molecules reaching odor receptors was constant. However, we can only assume that the odor stimuli given to participants were equal but there may have been some who sniffed the odor, thus getting more sensory neurons activated, which may have caused the neurons to adapt earlier [35,36]. Furthermore, as we could not control nor measure the depth of inspiration, the consistency of inspired volume cannot be assumed.

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