Brain and behavioral effects of swallowing carbonated water on the human pharyngeal motor system

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Gastrointestinal Centre, Institute of Inflammation and Repair, Manchester Academic Health Sciences Centre (MAHSC), University of Manchester, Salford Royal NHS Foundation Trust, Salford, United Kingdom; and Department Pädagogik und Rehabilitation, Ludwig-Maximilians-Universität München, Munich, Germany

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Elshukri O, Michou E, Mentz H, Hamdy S. Brain and behavioral effects of swallowing carbonated water on the human pharyngeal motor system. J Appl Physiol 120: 408–415, 2016. First published November 25, 2015; doi:10.1152/japplphysiol.00653.2015.—Chemical stimulation of the swallowing network with carbonation and citric acid has been investigated, showing potential benefits on swallowing of dysphagic patients. Despite this, the underlying mechanisms for these effects are not fully understood. Here we investigated the effects of 5 ml liquid bolus swallows of carbonated, citric acid, and still water on a swallowing reaction-time tasks paradigm in 16 healthy adults (8 male, mean age 33 ± 3.7 yr, protocol 1). We then investigated the net effects of “sensory bolus interventions” (40 repeated swallows every 15 s) of the three different liquid boluses on corticobulbar excitability, as examined with single-pulse transcranial magnetic stimulation (TMS) in 16 participants (8 female, mean age 33 ± 3.7 yr, protocol 2). The findings showed that a larger number of correctly timed swallows (within a predetermined time window) was accomplished mainly with carbonated liquids (z = −2.04, P = 0.04 vs. still water, protocol 1). Both carbonated and citric acid liquid interventions with 40 swallows increased corticobulbar excitability of the stronger pharyngeal projection, suggesting a similar modulatory pathway for the effects on swallowing. However, carbonation showed superiority (P = 0.04, F = 4.75, 2-way ANOVA), with the changes lasting up to 60 min following the intervention. These results hold significance for future further and in-depth physiological investigations of the differences between different stimuli on swallowing neural network.

A diffuse neuroanatomical network governs the sensorimotor act of deglutition (12, 24, 30, 41), and the importance of peripheral stimuli for the coordinated execution of this process is well characterized (10, 23, 40). It is now recognized that sensory input relayed to the swallowing centers is crucial for the initiation and modulation of the swallowing response. Previous neuroimaging and neurostimulation studies have shown that excitation of swallow-related representation in motor cortices (M1) can be modulated differentially with different bolus characteristics, such as taste and temperature (1, 39, 42). Altering the properties of peripheral stimuli forms part of various rehabilitation regimens during rehabilitation for dysphagia and is used as a means to increase sensory input in the oropharyngeal area (27).

Studies with neurostimulation, and particularly transcranial magnetic stimulation (TMS), have shown that corticobulbar excitability can be modulated after 10 minutes swallowing of glucose and quinine liquid boluses, with the changes lasting 30 min post-swallowing tasks (29). In patients with cerebral lesions, changes in swallowing speed were reported after swallowing of cold liquid boluses of citric acid (13) or in shortening the delay of pharyngeal swallowing response (22). Compared with water boluses, liquid boluses with citric acid also reduced swallowing speed in healthy adults (6). Work on animal models showed that the underlying mechanism for change is the increase of sensory input to swallowing centers via sensory nerves of the oropharynx (20).

Moreover, carbonation as a chemical sensation has also been used to induce behavioral changes. Earlier evidence showed that carbonation may increase the somatosensory perception by adding chemesthetic stimulation to the flavor/texture experience during water swallowing (8). Indeed, the behavioral effects of swallowing carbonated liquids have been examined by a number of groups (swallowing timing: 3, 7, 9, 36). More recently, studies have investigated the effects of carbonation on swallowing biomechanics and safety in dysphagic patients (4, 35) and swallowing performance with a swallowing reaction timed task (26).

Despite this increasing knowledge, the underlying mechanisms for the effects of sensory stimulation on swallowing, in addition to the duration of these effects, are yet to be fully understood. Both carbonation and citric acid liquid boluses have attracted great attention in rehabilitation studies. There is some evidence that the sensory input from the periphery is increased with these two stimuli.

Therefore, it would be of interest to investigate and compare the different effects of the different sensory stimuli on swallowing performance and corticobulbar excitability in health. We hypothesized that the increased somatosensory input of a “sensory bolus intervention” with both carbonated and citric acid liquid solutions would provoke changes in excitation of pharyngeal corticobulbar projection compared with still water in a healthy cohort. In addition and based on the aforementioned observations in the literature, sensory intervention with carbonation and citric acid would differentially modify swallowing latencies, resulting in quicker swallows and an increase in the number of successful challenged swallows in swallowing reaction tasks compared with intervention with non-carbonated solutions, with the use of a previously published methodology, the swallowing reaction time tasks (28). We decided to perform the studies in a healthy population prior to the use in the dysphagic population. Thus our participants’ (proprioceptive) sensory feedback loop (to cortical levels) would be intact. To further ascertain and verify the integrity of this sensory feedback loop in health and in the absence of standardized assessments, we also measured the sensory and tolerance pharyngeal
electrical thresholds with pharyngeal electrical stimulation. Thus we hypothesized that any changes in our measurements would be due to the sensory bolus intervention and not due to other noncentral sensory changes.

MATERIALS AND METHODS

Participants

Healthy participants were recruited following reporting no significant illnesses and their general practitioners were informed of their participation prior to the commencement of the study. Written informed consent was obtained from all participants before the experiments. All experiments were undertaken in the clinical laboratories of the Gastrointestinal Sciences at Salford Royal Hospital NHS Trust, United Kingdom, in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The approval for the studies was granted by the North West Research Ethics Committee.

The exclusion criteria included a history of epilepsy, previous brain or throat surgery, cardiac pacemaker, prior history of swallowing difficulty, neurological disease, pregnancy, the presence of metal implants in eyes or head, or intake of any medication that acts on the central nervous system or gastrointestinal tract.

Participants were enrolled in a series of experiments (4 arms per protocol, see below), all of whom provided written informed consent, being assigned to the two different protocols. Different participants were assigned to the two protocols, which run simultaneously.

In protocol 1, following a power calculation based on a previous study (26), 16 healthy participants [8 male, mean age 33 ± 3.7 (SE) yr, range 20–61 yr of age] participated.

In protocol 2, 16 healthy participants [8 female, mean age 33 ± 3.7 (SE) yr, range 21–60 yr of age] participated in this study.

Procedures

Pharyngeal electromyography (EMG) recordings. Participants were required to swallow a 3.2-mm-diameter intraluminal catheter (Gaeltec, Dunvegan, Isle of Skye) housing a pair of bipolar platinum ring electrodes. The tube was inserted either orally or transnasally according to subjects’ preference. An earth wire was connected to a skin electrode sited over the upper part of one of the sternocleidomastoid muscles in the neck. This catheter was connected with a preamplifier, amplifier, and interface (Cambridge Electronic Design, Cambridge, UK) to a laboratory computer and on which the Signal Application Program (Cambridge Electronic Design) allowed real time visualization and recording of the traces. This had filters set at 200 Hz to 2 kHz and allowed a sampling rate of 4–8 kHz. Signal software (Cambridge Electronic Design) was used for analysis of the amplitude and latencies of the traces.

Pharyngeal sensory and tolerance levels measurements. The sensory and tolerance levels were recorded following pharyngeal electrical stimulation (PES), delivered via the same intraluminal catheter used for the EMG recordings. The intraluminal catheter was connected to an electrical stimulator (Digitimer model DS7, Welwyn-Garden City, UK) and a trigger generator (Digitimer Neurology system). The sensory threshold was defined as the first perceptible sensation of electrical stimulation and was calculated as the average of three trials. For each trial, stepwise increments of ~0.1 mA/s were instigated starting at zero stimulator output. The maximum tolerated intensity was determined in an identical manner but this time the subject was asked to identify the point when the stimulation became uncomfortable such that they did not want the stimulation level to be increased any further. An earth wire was connected to a skin electrode sited over the upper part of one of the sternocleidomastoid muscles in the neck.

Preparation for solutions. Three different liquid boluses were used as a sensory bolus intervention: 1) carbonated solutions, 2) noncarbonated solutions (mineral water), and 3) solutions with weak acidity (citric acid). All solutions were kept at 6°C. The pH level of all solutions was examined prior to the start of each experiment with the pH meter (Jenway model 3310, Jenway, Gransmore Green, UK). The carbonated solutions were prepared by the investigators prior to each study, by adding 8 mg of CO₂ from a canister in 1 liter of water in a commercially available soda maker (SiSi, Siphon Soda-Seltzer maker), which holds the water at constant temperature (4°C), pH (4.1), and pressure (~60 bars/900 psi, resulting in 9 bars working pressure in a 1-liter bottle). The carbonated water was free of additional elements existing in commercially purchased soda beverages. The citric acid solutions were prepared by adding 50 mg (0.05 g) citric acid powder (commercially available from Westmill Foods, Enfield, Middlesex, UK) to 1 liter of water (pH level of 4.1), similar to the pH level of the carbonated solution. The mineral water was placed in glass containers of 1 liter prior to the study at the temperatures of 6°C. The temperature of the solutions in glass containers were constantly checked with lab thermometers throughout the studies.

“Swallowing reaction time” paradigm. The 3.2-mm intraluminal catheter used for EMG recordings and PES housed built-in pressure transducer between a pair of platinum ring electrodes (Gaeltec). The catheter was connected via a pressure measurement interface (Dept. of Medical Physics, Salford Royal Hospital NHS Trust) to a desktop computer running visual Basic software. Signals were digitally converted and monitored. Solid gel, cloth-backed disposable skin electrodes (H69P, Tyco Healthcare) were placed, 2 cm apart, to the dorsum of each volunteer’s hand and used to deliver an electrical pulse (cue) to the subjects through an electronic pulse generator (Digitimer model DS7; Welwyn-Garden City, UK) set to trigger at 7-s intervals. The subjects were asked to perform swallows of 5-ml boluses of the liquids delivered into the mouth via a plastic catheter connected to a hand-held syringe.

Swallowing reaction time (SRT) was calculated as the latency between the delivery of the electrical cue to the onset of the pharyngeal swallow, registered via the pressure signal crossing a predefined pressure threshold (~50% of the pressure amplitude to 6 single calibration swallows). The subjects had to swallow the different boluses following electrical cues delivered to the thenar muscle as follows: 1) at their own pace (termed normal swallows); 2) at a fast pace; and 3) during a predetermined 150-ms time window (challenged swallows), calculated from the difference in the latencies of the normal and fast swallows according to the formula:

\[
\text{Challenge Time Window} = \text{Fast SRT} + \left( \text{Normal SRT Time Window} - 2 \right) + 75 \text{ ms}
\]

The SRT and the successful (swallows within the predetermined challenge time window) and unsuccessful challenged swallows (swallows before or after the predetermined time window) were recorded by “Swallow Splash” Software (Dept. of Medical Physics, Salford Royal Hospital NHS Trust) for further off-line analysis.

Taste intensity and taste difference scales. To assess the intensity of perception of taste, at the end of protocol 1, each subject held each bolus in their mouth for 3 s tasting the samples and then rinsed his or her mouth twice before the next bolus. After each swallow, both the Labeled Magnitude Scale (LMS) (11) and difference test chart were presented to the participants to identify taste, the degree of difference, and its intensity. The LMS includes seven verbal labels arranged according to their magnitude (nothing, barely detectable, weak, moderate, strong, very strong, and strongest imaginable sensation). The difference test chart was divided into five levels (no difference = 1, very mild difference = 2, mild difference = 3, moderately different = 4, extremely different = 5).

Transcranial magnetic stimulation. Focal TMS was performed using a flat figure-of-eight shaped magnetic coil (outer diameter, 70 mm) connected with a Magstim BiStim2 magnetic stimulator (Magstim, Whitland, Wales, UK), which produced maximal output of 2.2 T. The anterior-posterior direction with the plane of the coil parallel to the scalp surface and the handle/axis of the coil at 45° to mid sagittal line was
chosen according to previous studies. TMS was used to localize the exact resting pharyngeal motor cortex (M1) hotspots bilaterally. The hotspots were identified as the area where the minimum intensity of stimulator output was required to achieve motor evoked potentials (MEP) of at least 20 μV in 5 of 10 trials. The stronger pharyngeal projection hotspot was the hemispheric site evoking the greatest pharyngeal response at the lowest threshold and the weaker pharyngeal projection hotspot was the hemispheric hotspot for pharyngeal musculature on the contralateral cortex. MEP amplitude at follow-up time points was assessed with TMS pulses at 110% and 120% of pharyngeal motor threshold, with 20 stimuli being given at these intensities and repeated bilaterally after the intervention.

*Thenar EMG recordings.* Solid gel, cloth-backed disposable skin electrodes (H69P, Tyco Healthcare, UK) were placed 1.5 cm apart over the Abductor Pollicis Brevis muscle contralateral to the hemisphere giving the largest pharyngeal MEP (PMEP) following single TMS pulses. A corresponding earth was attached to a skin electrode on the wrist. The equipment and the software used for the pharyngeal measurements were also used to obtain and analyze thenar MEP (TMEPs). The same procedure as for PMEPS was followed for the identification of the MT for thenar muscle with TMS, defined as the intensity producing TMEPs of at least 50 μV on at least 5 of 10 consecutive occasions, which served as a control for the study. MEPS were recorded for further analysis using disposable electrodes placed over the hand opposite the side of the brain evoking the largest pharyngeal response, and then the optimal site and motor threshold for thenar stimulation was identified as described above.

**Experimental Protocols**

*Protocol 1: effects of sensory bolus interventions on swallowing behavior.* In protocol 1, subjects attended the laboratories on four separate visits at least 3 days apart. On the first three occasions, participants received each of one of the sensory bolus interventions: carbonated, citric acid, and noncarbonated liquids (mineral water) in a randomized order. Each participant was asked to attend the laboratory on one last occasion to complete two scales: taste intensity and taste difference. On each of the first three visits, a pharyngeal EMG-pressure transducer catheter was inserted using the established pull-through technique, allowing for direct assessment for the upper esophageal sphincter’s resting pressure when passed in the oropharynx. With the catheter in situ at the midline pharyngeal area, the sensory and maximum tolerance thresholds were recorded following PES, before performing a swallowing reaction time paradigm. These measurements were used as a baseline on each visit and were compared within participant. The rationale for performing these measurements prior to each protocol was that PES threshold would show if there was any change in sensation for each participant between days, which could play a role in the results or affect the results of the sensory interventions. Subjects were asked to perform baseline measurements of swallowing behavior (comprising 30 swallows: 10 normal, 10 fast, and 10 challenged swallows within a predetermined time window) as described in Procedures with the sensory bolus intervention liquids randomized for the day. Each solution was delivered to the subject’s mouth manually using a single-use plastic syringe and a small plastic single-use tube. A straw was attached to the tube and the subjects were asked to hold the straw between the lips, approximately in the midline of the mouth. Swallowing reaction times and challenged swallows were performed at baseline, and then at 15, 30, 45, and 60 min after the baseline recordings with each solution on each randomized visit.

*Protocol 2: effects of sensory bolus interventions on corticopharyngeal excitability.* Subjects attended the laboratory on three separate visits at least 3 days apart. On each occasion they received each of the same sensory bolus interventions as described in protocol 1 in a single-blinded randomized manner. On each visit, they sat in a comfortable chair and a pharyngeal EMG catheter was inserted following the manometric procedures as in protocol 1. Solid gel, cloth-backed disposable skin electrodes (H69P, Tyco Healthcare, UK) were placed 1.5 cm apart over the Abductor Pollicis Brevis muscle contralateral to the hemisphere giving the largest PMEP. A corresponding earth was attached to a skin electrode on the wrist. A disposable surgical cap was put on the head and taped to allow for the marking of the cranial vertex following identification, as previously described (17). PES was again used to evaluate the sensory and tolerance thresholds, and the baseline of MEPS as described in Procedures were obtained for both the pharynx and hand (thenar) using single-pulse TMS. The participants then received the sensory bolus intervention. The intervention included the delivery of 3-ml boluses intraorally via a plastic catheter connected to a hand-held syringe, one every 15 s (40 swallows in total). A visual cue was presented to them on a personal computer to instruct when to swallow. Following the interventions, the neurophysiological measurements were repeated with single-pulse TMS immediately, and after 15, 30, 45, and 60 min postintervention.

**Data Analysis**

*Protocol 1.* Pharyngeal sensory and tolerance threshold were compared with nonparametric statistics across all different days. Nonparametric tests (Friedman’s test) and intraclass correlation (ICC, single measures) for the five sets of data collected at baseline, 15, 30, 45, and 60 min postbaseline were used to investigate variability and agreement across the measurements of the different study runs for each of the solutions and for each swallowing paradigm (normal, fast, challenged swallows) (SPSS 16.0). Specifically for the challenged swallows, we counted the number of successful swallows for each run (out of 10 swallows), and the percentage change of the grand mean average of five repeated runs of timely executed swallows for each volunteer and for each solution was then calculated.

In the case of significant differences in the distributions, Wilcoxon’s test was then used to assess the difference. Taste intensity difference from the LMS and taste difference scales between still water, carbonated solution, and citric acid solution was also tested with Wilcoxon’s test. *P* values of <0.05 were taken as a measure of statistical significance, and data are expressed as means (±SD) unless stated otherwise.

*Protocol 2.* The peak-to-peak amplitude of PMEPS evoked by TMS was used as a measure of motor cortex excitability. Signal software was used to review individual PMEPS in microvolts and to identify the amplitude of each trace. Ten MEPS were recorded from each of the stronger, weaker, and thenar representations. Therefore 30 MEPS from baseline and each time point were used for subsequent analysis. The average amplitude of both intensities (110% and 120%) for each time interval was calculated. After that, grand mean amplitudes for each time point were calculated for each individual and normalized to the baseline and were shown as a percentage change from baseline. SPSS 14 (SPSS, Chicago, IL) was used for the statistical analysis of the normalized data. Comparison was made between the different interventional groups for the cortical excitability changes for each hemispheric hotspot. The GLM repeated-measures ANOVA was used, including each time point except the baseline. The data were expressed as means (±SE) unless stated otherwise, and *P* values of <0.05 were taken as a measure of statistical significance. Across the different study days, the reproducibility of baseline raw data was investigated using nonparametric tests (Friedman’s test).

**RESULTS**

*Protocol 1: Effects of Sensory Bolus Interventions on Swallowing Behavior*

All participants completed the study without any side effects. The baseline sensory and tolerance thresholds across all three studies were similar across all studies (Friedman’s test:
sensory threshold: $\chi^2 = 0.667, P = 0.717$; tolerance threshold $\chi^2 = 3.500, P = 0.174$). The sensory threshold ranged from 1.11 to 3.52 mA, whereas the tolerance threshold ranged from 2.72 to 7.71 mA.

Table 1 presents the group grand mean of response latencies in milliseconds for the normal and fast swallows and the grand means of the successful swallows across all three sensory bolus interventions across the baseline and the follow-up measures.

**Normal swallowing reaction times.** For still water ($\chi^2 = 1.1, P = 0.8$), carbonated solutions ($\chi^2 = 2.7, P = 0.6$), and citric solutions ($\chi^2 = 4.6, P = 0.3$), there were no significant differences across the different time points, and the intraclass correlation coefficient (ICC) was high within each of the “sensory solutions.” For mineral water swallowing trials, ICC indicated almost perfect agreement [ICC: 0.9, 95% confidence interval (CI): 0.951–0.991], while for the trials with solutions of carbonated and citric acid, the identical repetitions were in excellent agreement, with ICC reaching 0.9 (95% CI: 0.844–0.970) for carbonation and 0.9 (95% CI: 0.591–0.921) for citric acid, respectively. To test the difference between different solutions, we calculated the grand mean of all time points and compared the distributions of the latencies for the different solutions. However, no significant difference was found ($\chi^2 = 0.9, P = 0.6$).

**Fast swallowing reaction times.** Using the Friedman’s test, the latencies across the five time points within each of the three different solutions were not different (mineral water: $\chi^2 = 3.4, P = 0.4$; carbonated: $\chi^2 = 6.9, P = 0.1$; and citric acid $\chi^2 = 4.6, P = 0.3$). ICCs also showed high agreement across sessions, similar to still water. Comparison of the grand mean latencies across all time points between the three solutions showed no significant difference ($\chi^2 = 0.5, P = 0.7$).

**Challenged swallows.** Using Friedman’s test, the number of successful swallows for the five time points for each of the three solutions for the challenged swallowing tasks were similar (still water: $\chi^2 = 3.1, P = 0.6$; carbonated solution: $\chi^2 = 7.1, P = 0.1$; citric acid solution: $\chi^2 = 2.7, P = 0.6$). Intraclass correlation for still water swallowing reaction time was 0.732 (95% CI: 0.255–0.657), for carbonated solution was 0.599 (95% CI: 0.146–0.7), and for citric acid solution was 0.808 (95% CI: 0.591–0.921). These results indicate a good agreement for all three solutions.

The mean swallowing percentage of successful swallows for the carbonated solution was higher than that of still water and citric acid solution. Friedman’s test showed a significant difference in the distributions between the three solutions ($\chi^2 = 6.054, P = 0.048$). A significant difference between the mean successful swallows of carbonated and still water was observed ($z = -2.04, P = 0.04$), but no significant difference between successful timed challenged swallow tasks of carbonated solutions and citric acid were observed ($z = -1.293, P = 0.196$) or between citric acid solution and still water ($z = -0.04, P = 0.96$) (Wilcoxon’s tests).

**Intensity and difference rating.** The different intensity scores for each taste on the intensity LMS were statistically analyzed with nonparametric tests. The highest intensity perception by the participants was scored for carbonated solutions and was followed by citric acid solutions. The mean intensity rating for water was close to zero. Wilcoxon’s test showed a significant difference between the intensity scores for carbonated solutions and the other two solutions (water and citric acid) and significant difference between still water and citric acid solution [carbonated solution vs. still water ($z = -3.51, P < 0.001$), carbonated solution vs. citric acid solution ($z = -3.52, P < 0.001$), and citric acid solution vs. still water ($z = -2.20, P = 0.028$)]. The results from the “difference” scale were not similar. The carbonated solution was mostly perceived as “extremely different” compared with still water and citric acid solutions, and all participants perceived the difference between carbonation and other solutions ranging from extreme to mild. However, the participants commented that they did not perceive any difference between water and the citric acid solution, while three subjects scored this difference as very mild and one subject found only a mild difference.

**Protocol 2: Effects of Sensory Bolus Interventions on Corticopharyngeal Excitability.**

All participants completed the study without any side effects on three different days. The baseline sensory and tolerance thresholds during the three studies were similar for each participant (Friedman’s test: sensory threshold: $\chi^2: 4.500, P = 0.105$; tolerance threshold: $\chi^2: 1.625, P = 0.444$). The minimum (sensory) threshold ranged from 1.3 to 7.8 mA whereas the maximum (tolerance) threshold ranged from 2.5 to 23.3 mA. The stronger corticobulbar pharyngeal projection was found on the right hemisphere in 12 of 16 participants. The stronger corticobulbar pharyngeal projection was between 5.1 ± 0.5 cm (mean ± SD) anterior to the vertex and 2.3 ± 0.4 cm (mean ± SD) lateral to the midline for the right hemisphere. In subjects who showed left hemispheric stronger pharyngeal representation, the optimal hotspot was between 4.7 ± 1.1 cm (mean ± SD) anterior to vertex and 2.5 ± 0.4 cm anterior to the vertex and 2.3 ± 0.4 cm lateral to the midline.

### Table 1. Swallowing reaction times with different stimuli

<table>
<thead>
<tr>
<th>Swallow Type</th>
<th>Water Swallow Condition</th>
<th>Baseline</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Grand Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, ms</td>
<td>Carbonation</td>
<td>1,455 ± 69</td>
<td>1,470 ± 82</td>
<td>1,500 ± 92</td>
<td>1,526 ± 83</td>
<td>1,491 ± 91</td>
<td>1,489 ± 87</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1,451 ± 95</td>
<td>1,487 ± 103</td>
<td>1,451 ± 114</td>
<td>1,427 ± 107</td>
<td>1,435 ± 96</td>
<td>1,451 ± 103</td>
</tr>
<tr>
<td></td>
<td>Mineral</td>
<td>1,488 ± 111</td>
<td>1,536 ± 109</td>
<td>1,512 ± 100</td>
<td>1,518 ± 109</td>
<td>1,478 ± 103</td>
<td>1,507 ± 107</td>
</tr>
<tr>
<td>Fast, ms</td>
<td>Carbonation</td>
<td>1,000 ± 70</td>
<td>1,018 ± 73</td>
<td>1,018 ± 81</td>
<td>954 ± 65</td>
<td>920 ± 67</td>
<td>982 ± 71</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1,053 ± 69</td>
<td>992 ± 78</td>
<td>993 ± 76</td>
<td>946 ± 86</td>
<td>898 ± 82</td>
<td>976 ± 78</td>
</tr>
<tr>
<td></td>
<td>Mineral</td>
<td>1,090 ± 86</td>
<td>999 ± 74</td>
<td>955 ± 63</td>
<td>933 ± 60</td>
<td>962 ± 89</td>
<td>988 ± 74</td>
</tr>
<tr>
<td>Challenged swallows, %</td>
<td>Carbonation</td>
<td>35 ± 4</td>
<td>47 ± 4</td>
<td>45 ± 3</td>
<td>41 ± 3</td>
<td>51 ± 5</td>
<td>45 ± 4</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>38 ± 4</td>
<td>43 ± 5</td>
<td>39 ± 5</td>
<td>43 ± 4</td>
<td>38 ± 4</td>
<td>40 ± 4</td>
</tr>
<tr>
<td></td>
<td>Mineral</td>
<td>35 ± 3</td>
<td>42 ± 4</td>
<td>36 ± 3</td>
<td>46 ± 5</td>
<td>45 ± 4</td>
<td>41 ± 4</td>
</tr>
</tbody>
</table>

Mean and grand mean latencies (mean ± SD) for repeated trials for normal, fast swallows, and successful challenged swallows (% with carbonated, acidic, and mineral solution swallows by healthy adults. Carbonation increased the percentage of successful swallows compared to mineral water ($z = -2.04, P = 0.04$).
lateral to the midline. The average TMS intensity used for elicitation of PMEPs from the stronger M1 was 61.9 ± 1.2% (mean ± SE), whereas from the weaker hemisphere it was 63.5 ± 1.2% (mean ± SE). The average of TMS intensity from the thenar representation was 39.5 ± 1.2% (mean ± SE). Baseline pharyngeal and thenar excitability prior to the experiments was similar across all the study days for each site: stronger and weaker hemispheric representation and thenar cortical representation (Table 2, Friedman’s test, stronger pharyngeal: $\chi^2 = 2.6, P = 0.26$; weaker pharyngeal: $\chi^2 = 2.13, P = 0.34$; thenar: $\chi^2 = 1.6, P = 0.44$).

**MEP amplitudes.** The group mean percentage change in PMEPs amplitudes of the stronger corticobulbar pharyngeal projection from the baseline of the 16 subjects after the different types of swallowing intervention at the different time points are shown in Fig. 1A.

A three-way ANOVA was used to analyze data with factors of intervention (carbonated, water, and citric acid), time (immediately, 15, 30, 45, and 60 min) and site (stronger and weaker pharyngeal and thenar representation). There was a significant intervention $\times$ time $\times$ site interaction $[F(1,15) = 4.63, P = 0.04]$, which allowed for a 2-way ANOVA within the stronger pharyngeal MEPs and showed a significant interaction time $\times$ intervention interaction $[F(1,15) = 4.75, P = 0.04]$. By contrast, there was no significant time $\times$ intervention interaction in the weaker pharyngeal projection $[F(1,15) = 0.89, P = 0.36]$ (Fig. 1B). Hence, focusing on the stronger projection, there was also a significant effect of intervention $[F(1,15) = 9.440, P = 0.008]$. The increase in excitability from the stronger corticobulbar projection following the carbonated solution intervention showed the highest values at 45 min (62.12 ± 21.31%) and at 60 min (61.31 ± 26.31%) from the baseline (paired $t$-test, $P < 0.05$).

By comparison, there was no significant interaction for thenar MEPs with a two-way ANOVA showing no effect for the different interventions on thenar excitability $[F(1,15) = 0.279, P = 0.609]$.

**MEP latencies.** There was no significant difference in the MEP latencies across the different sensory interventions and time points (3-way ANOVA).

**DISCUSSION**

We investigated the effects of a sensory bolus intervention, with three different chemesthetic modalities, namely, carbonation, citric acid, and still water repetitive swallowing, on corticobulbar excitability and swallowing reaction time tasks in healthy participants. The results from our studies only partially supported our hypothesis that both carbonated and citric acid solutions would have a similar modulatory profile. Instead, we found that carbonation allowed for a higher percentage of successful swallows compared with still water. We also found that both sensory interventions with carbonation and citric acid solutions showed increases in corticobulbar excitability; however, only the sensory intervention with carbonated boluses showed statistical significant changes on corticobulbar excitability lasting up to 60 min following the intervention. Moreover, the carbonated solutions were perceived as "extremely different,” with “higher intensity” compared with the citric acid, and still water repetitive swallowing, on corticobulbar excitability and swallowing reaction time tasks in healthy participants. The results from our studies only partially supported our hypothesis that both carbonated and citric acid solutions would have a similar modulatory profile. Instead, we found that carbonation allowed for a higher percentage of successful swallows compared with still water. We also found that both sensory interventions with carbonation and citric acid solutions showed increases in corticobulbar excitability; however, only the sensory intervention with carbonated boluses showed statistical significant changes on corticobulbar excitability lasting up to 60 min following the intervention. 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acid boluses and still water. Thus our results are worthy of further discussion.

The intervention with carbonated solutions compared with still water showed similar results to previous reports on swallowing reaction times tasks (26). In both studies, carbonation did not have an effect on either normal or fast swallowing latencies, rather only on the percentage of successful swallows within a predetermined time window. Although in our previous study we adopted a pseudorandomized order to examine the effects of carbonated solutions compared with still water (ABAB, etc.), here we also investigated the repeatability of the effects by repeating the intervention over time. Interestingly enough, the results showed that the effects of carbonation are reproducible over time, and the reproducibility measures (intraclass correlations) were similar to the ones we observed previously (26). However, it is important to note that the confidence intervals following carbonated and citric acid liquid swallowing were wider when visually compared with the confidence intervals of water swallowing. Wider confidence intervals may imply that the sample was not large enough to draw firm conclusions. However, given that the CI for the water swallows was relatively good, we must speculate that within our population of healthy participants, the sensory input from carbonated and to a greater extent citric acid liquids resulted in heterogeneity in responses. Replication of the study with a larger sample will provide some insight into the mechanisms that could account for this observation.

In the literature, there are several reports on the beneficial effects of carbonation on swallowing in neurogenic dysphagia (4, 19, 35), and our study contributes to the number of studies showing that carbonated solutions can be beneficial for the swallowing network. Such a pronounced effect of carbonated solutions on swallowing is suggestive of the fact that carbonation does not act only on the taste system (5), but also in other orosensory pathways, as previously suggested (21, 37). This “increased intensity” of sensory information with carbonation which is relayed to the higher cortical centers involved in swallowing might explain why only challenged swallows were benefited. The superiority of carbonated solutions in inducing changes in swallowing and the direct link to the cortical centers is verified by the results from our second protocol. The net effect of the intervention with carbonated solutions produced an increase of M1 corticobulbar excitability compared with still water and solutions with citric acid, as discussed below.

It was also interesting to observe that intervention regimen with citric acid solutions showed positive changes in cortical excitability examined with TMS. The two sensory bolus interventions (carbonation and citric acid) were carefully designed so that the level of acidity was equal (pH 4.1) for both boluses over the different days. The similar outcome is perhaps suggestive of a shared underlying mechanism which we speculate is the acidity leading potentially to activation of lingual nociceptors exciting trigeminal neurons in turn, and signaling oral irritation to higher centers involved in swallowing and taste central brain regions which overlap (32, 39). In support of our argument, the recent animal studies (5) showed that sour cells on the tongue provide the cellular sensors for carbonation. Our data corroborated this suggestion in healthy adults. Further support and corroboration of our results in turn are the results from fMRI studies showing that lemon-flavored liquid stimulation increased the cortical activation in the swallowing neural network (sensory-motor cortex, insula, cingulate gyrus, prefrontal cortex) compared with saliva and water swallows unilaterally (1) and bilaterally (16). Last, it should be mentioned that for both the carbonation and citric acid interventions, the effects in the healthy participants population were observed only in the “challenged” task mode with the predetermined time window, which is perhaps due to the fact that this task included was more challenging compared with the otherwise normal and safe swallow of the healthy participants in our study.

Even though both sensory bolus interventions employed similar acidity and increased the percentage of successfully timed swallows, the participants in our study commented that the taste intensity was different for the different boluses. Participants found that the carbonation boluses were “highly different” compared with the other two solutions. Perhaps one of the differences in this perception was the effervescence of carbonation and the unique chemesthesis it provides. This difference between the otherwise equal-in-acidity solutions is potentially the underlying reason that sensory bolus intervention with carbonation resulted in increased cortical excitability of the stronger pharyngeal projection compared with the interventions with citric acid solutions and still water. The heightened perception of the sensory stimuli with carbonation could have resulted in increasing cortical excitability further compared with citric acid liquid swallowing. In addition, the literature suggests that both mechanoreceptors and nociceptors are activated with carbonation, while the feeling of “overall fizziness” of carbonated solutions relates to the sensation of the bubbles bursting activating the mechanoreceptors (15). Interestingly, the cortical excitability was only statistically significant for the stronger pharyngeal projection, even though bilateral changes were observed. Moreover, our data from our control site (thenar representation) showed that the cortical increase in excitability was “intervention specific.” A further important point of discussion is the increase of cortical excitability with peaks at 30 and 60 min compared with water and citric acid solutions (Fig. 1B), which is indicative of the fact that changes can develop and can be sustained for longer than the duration of the intervention. Our results have several implications and are of importance for the utilization of the sensory bolus interventions in the rehabilitation of swallowing disorders. Further work in dysphagic patients with neurophysiological measurements remains to be done to provide insight into the underlying mechanism.

One of the limitations in our study was not examining for the other factors affecting individual taste perception such as genetic taste-status factors (supertasters vs. nontasters) (2, 34). Recently it has been observed that the perceived intensity of a taste stimulus varies as a function of stimulus concentration, taste quality, participant age, and genetic taste status, and influences swallowing pressure amplitudes (31). Here, we tried to keep the acidity levels equal between the carbonated and citric acid boluses, and therefore we might not have used a concentration of acidity in the citric acid sensory intervention allowing for heightened changes in swallowing. In other published research, where citric acid boluses show the greatest effect on a lingual strength measurement compared with carbonation, a concentration of 2.7% wt/vol was used (33). However, in our protocol we wanted to elucidate the direct difference between citric acid and carbonated sensory bolus...
interventions accounting for the factor of acidity. Future work should explore how the intensity of a stimulus and the degree to which a participant likes or dislikes the stimulus could have affected the results. It might well be that the continued application of effervescence in the swallowing intervention of 40 swallows in total could have resulted in increased unpleasantness which then heightened cortical excitability. Our work differs from other research studies where much fewer single swallows (up to 4 in total number) were trialed (33) and as such may have created a more sustained effect not seen in other reports. In addition, the extent and the duration of any activation in the bulbar swallowing network were not examined in our study. We investigated the effects of sensory stimulation on the corticobulbar pathway; however, investigating the effects of the sensory input in the bulbar network would allow us to understand the underlying mechanisms further. Future studies may benefit from the investigation of the trigeminal-bulbar pathway excitation as described previously (12, 18, 25). Last, we did not include a sham arm in our study, but only a control arm with water swallowing of similar intensity and frequency to that of carbonation and citric acid solutions. Although there is evidence from previous studies showing that cortical excitability is stable over time and single TMS pulses do not increase cortical excitability (38), future studies would benefit from a control arm with no swallowing, which might provide clear evidence for the corticobulbar excitability of the participants over time.

In conclusion, we showed that sensory intervention with boluses of different chemesthetic properties but with the same acidity, such as liquid boluses of carbonated and citric acid can increase cortical excitability compared with intervention with still water swallows. However, only the swallowing of carbonated liquids showed a statistically significant increase. Moreover, only carbonated boluses showed an increase in the number of correctly timed swallows in healthy subjects. Future work is warranted for the means and the proper administration of the intervention in dysphagic patients prior to investigating whether the results from this study in normal and safe swallowing are corroborated by investigations in patients’ swallowing impairments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


