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DOI:

[10.1111/nmo.14286](https://doi.org/10.1111/nmo.14286)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Michou, E., & Hamdy, S. (2021). Reversal of the effects of focal suppression on pharyngeal corticobulbar tracts by chemesthesis coupled with repeated swallowing. *Neurogastroenterology & Motility*, [e14286].
<https://doi.org/10.1111/nmo.14286>

Published in:

Neurogastroenterology & Motility

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Title

Reversal of the effects of focal suppression on pharyngeal corticobulbar tracts by chemesthesis coupled with repeated swallowing

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Article Type: Original Research

Running title: Chemesthesis reverses virtual lesion

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Abstract

Background: Previous reports suggested the potential benefit of chemesthesis in the form of carbonated water (CW) integrated within dysphagia rehabilitation protocols. Here, we examined the effects of CW within a repeated swallowing protocol following focal suppression to pharyngeal cortical representation as a prelude to its application in dysphagic patients. **Methods:** Fourteen healthy volunteers participated in a 3-arm study. Each participant underwent baseline corticobulbar pharyngeal and thenar motor-evoked potential (MEP) measurements with Transcranial Magnetic Stimulation (TMS). Subjects were then conditioned with 1Hz repetitive (r)TMS to induce focal unilateral suppression of the corticopharyngeal hotspot before randomisation to each of three arms with 40 swallows of CW, non-CW and saliva swallowing on separate days. Corticobulbar and thenar MEPs were collected for up to 1 hour and analysed using repeated measures (rm)ANOVA.

Results: A 2-way rmANOVA for Intervention x Time showed a significant effect of Intervention ($F_{(1,13)}=7.519$, $P=0.017$) in both ipsi- and contra-lesional corticopharyngeal projections. Carbonation showed superiority in facilitating change by increasing pharyngeal cortical MEPs compared to non-CW ($z=-3.05$, $P=0.002$) and saliva swallowing ($z=-2.6$, $P=0.008$). No change in thenar representation (control) was observed nor in MEP latencies from both pharyngeal and thenar musculature.

Conclusions: We conclude that interventional paradigms with CW have the capacity to reverse the effects of a focal suppression with 1Hz rTMS more strongly than non-CW or saliva swallowing alone, producing site specific bi-hemispheric changes in corticopharyngeal excitability. Our data suggest that carbonation produces the effects through a mainly cortical mechanism.

Introduction:

The importance of sensory input in the development of interventional protocols for dysphagia rehabilitation has been increasingly recognised in recent years. From a neurophysiological perspective, it has been hypothesised that sensory input activating receptors in the oral cavity, epiglottis, laryngeal and pharyngeal areas that reach the Nucleus Tractus Solitarius (NTS) of central pattern generator (CPG) will further increase the activation of higher cortical substrates via afferent connections [1]. Further evidence for the effects of sensory input on higher level swallowing neural circuitry has been gleaned from studies with different stimuli from the periphery [2-4], from studies incorporating neuroimaging and Transcranial Magnetic Stimulation (TMS) approaches [5-8] and clinical studies with patients suffering from dysphagia undergoing treatment [9-11]. This evidence has provided coherent information regarding the modulatory abilities of chemosensation and gustatory stimulation on the swallowing network.

Differences in either behavioural measures or swallowing biomechanics have been observed when introducing different tastants or other chemesthetic input to healthy participants and dysphagic patients. Apart from sour taste [2, 12-14] which implies the use of lower pH (4-4.1), carbonation has been also used as a chemesthetic stimulus for swallowing, based on earlier reports of increased somatosensory perception that adds to the flavour/texture experience during water swallowing [15]. A carbonated water bolus has been reported to decrease pharyngeal transit time [16] or increase electromyographic (EMG) spectra components [17-18], linguopalatal pressure during swallowing [19] and the success rate of challenged swallows in a swallowing reaction time task [4]. Moreover, studies have investigated the effects of carbonation on swallowing biomechanics and safety in dysphagic patients [9,16, 20-23] while comparing the effects of single sips of carbonated vs non-carbonated boluses on videofluoroscopic measurements or laryngeal EMG [24].

Recently, various physiological and neuroimaging techniques have also been utilised to elucidate whether the observed positive effects of carbonation are due to changes at different levels of central nervous system. Apart from swallowing reaction time tasks [4,6] and the direct comparison to citric acid and non-acidic boluses, studies have also observed marked changes on corticobulbar excitability of the pharyngeal representation evaluated with single-pulse Transcranial Magnetic Stimulation (TMS) lasting up to 60 minutes in 2 different studies [6-7].

Focal suppression delivered with low-frequency 1Hz rTMS usually serves as a model to replicate the cortical disruption seen in stroke patients with dysphagia [25-26]. One-Hz rTMS paradigm for 10 min can generate a unilateral 'virtual lesion' affecting cortico-bulbar output, in the pharyngeal motor cortex (PMC) for up to 45mins. This is coupled with interference in swallowing behaviour, as measured with reaction time swallowing tasks [27] and an observed transient change in swallowing behaviour that is reminiscent to that seen in stroke patients with hemispheric lesions [28].

Moreover, despite there being some evidence for the immediate changes in physiology with carbonated compared to non-carbonated boluses, the extent to which the chemesthetic stimuli can promote changes in the swallowing brain network is still under investigation. Hence, the aim of our study was to observe the ability of carbonated boluses introduced in specified interventional protocol to modulate cortical excitability in a perturbed system in healthy subjects after the induction of a focal suppression to PMC representation. We hypothesized that repetitive swallowing of carbonated boluses in a specified regime will reverse the cortical suppression in healthy subjects in manner superior to swallowing non-carbonate water or saliva alone.

Methods

Participants

Pre-screened healthy participants were recruited to the study. Written informed consent was obtained from all participants before the experiments. All experiments were undertaken in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The approval for the studies was granted by the Great Manchester (Central North West 7) Research Ethic Committee (10/H1008/61). The (pre-screened) 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.

Sample Size: Power calculations performed by the Medical Statistics department using information from previous published study [4,27-28] indicated that the number of participants required to allow appropriate statistical power (80%), alpha (0.05) was 12.

Experimental Procedures:

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 Company, UK), which produced a maximum output of 2.2 Tesla. The antero-posterior direction with the plane of the coil parallel to the scalp surface and the handle/axis of the coil at 45 to the midsagittal line was chosen according to previous studies [29].

Pharyngeal and Thenar EMG measurements:

Pharyngeal electromyographic (EMG) measurements after PMC stimulation with TMS, termed pharyngeal motor evoked potentials (PMEPs), were recorded through a 3.2 mm diameter intraluminal catheter (Gaeltec Ltd, Scotland), with a built-in pair of bipolar platinum ring electrodes, which was inserted either nasally (15-17 cm to pair EMG electrodes from the nasal flare) or orally (13-15 cm from the anterior incisors) depending on subject preference. This allowed recording of PMEPs at the mid-pharyngeal level adjacent the middle pharyngeal constrictors. During the measurements capture, an earth wire was connected to a skin electrode sited over the upper part of one of the sternocleidomastoid muscles in the neck to improve signal quality.

As a control, thenar EMG from the abductor pollicis brevis (APB) muscle contralateral to the hemisphere giving the largest EMG of PMP was also recorded by TMS. A pair of gel electrodes (H69P; Tyco Healthcare, Gosport, UK) was placed on the hand opposite the side of the brain, evoking the largest pharyngeal response to record the thenar motor-evoked potentials (TMEPs). An additional earth was connected to a skin electrode sited over a bony prominence on the wrist.

In order to record both muscle MEPs, the relevant electrodes were connected to a preamplifier (CED 1902; Cambridge Electronic Design, Cambridge, UK) with high- and low-pass filter settings of 200 Hz and 2 kHz, respectively, via connecting cables. Response signals were processed through a 50/60-Hz noise eliminator (HumBug; Quest Scientific, North Vancouver, BC, Canada) to remove any unnecessary electrical interference and collected through a laboratory interface (CED micro 1401; Cambridge Electronic Design) at a sampling rate of 5 kHz and recorded using Signal software (ver. 2.13; Cambridge Electronic Design) running on a personal computer.

For the pharyngeal MEPs:

Single-pulse TMS was used at the start of each study to determine the strongest pharyngeal cortical projection and to determine the optimal coil positions for recording PMEPs (motor hot

spots) over both hemispheres. The motor threshold (MT) was identified at that site using single TMS pulses to achieve PMEPs greater than 20 μ V in at least five of 10 trials. The PMC site which produced the largest amplitude of PMEPs, at the lowest threshold, was defined as the “stronger” pharyngeal projection, and the contralateral as the “weaker” pharyngeal site.

For the Thenar MEPs:

Thenar motor evoked potentials (TMEPs) from Abductor pollicis brevis (APB) muscle were also recorded from motor hotspots. For the identification of the Thenar resting MT (rMT) for thenar muscle, the TMS procedure was the same as with PMEPs. Initially, the site was specified and the intensity producing TMEPs of at least 50 μ V on at least 5 of 10 consecutive occasions was identified in order to set the rMT.

Focal Cortical Suppression:

To induce focal cortical suppression, or “virtual lesion” [27], trains of stimuli were delivered through the figure-of-eight coil connected to Magstim super rapid stimulator (Magstim Company, UK) with a maximum output of 1.8 T. The focal cortical suppression was created with 1Hz rTMS at 120% of pharyngeal MT (capped to a maximum of 100% of stimulator output) for 10 minutes with 600 single pulses, as previously described [27].

Pharyngeal Sensory Threshold:

In order to further ascertain and verify the integrity of the pharyngeal sensory feedback loop in health, we also measured the sensory and tolerance pharyngeal thresholds. The pharyngeal sensory threshold from the hypopharynx was recorded through the same catheter but connected to an electrical stimulator (Digitimer model DS7, Welwyn-Garden City, Herts, UK) and a trigger generator (Digitimer Neurology system, Herts, UK), allowing measurements of an individual’s sensory and maximal pharyngeal electrical thresholds (average of 3 trials). The sensory threshold

was defined as the first perceptible sensation of electrical stimulation felt by the participant in the throat. For each trial, stepwise increments of 0.1 mA/s were instigated starting at zero stimulator output. The pharyngeal 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.

Solutions preparation:

This protocol consisted of 3 different arms. Two different liquid boluses were used as the “sensory bolus intervention” [6] arms comprising: 1) carbonated solutions, 2) non-carbonated solutions (still water). A third arm was chosen to compliment the liquid bolus interventions using saliva swallowing alone. The pH level of both solutions was examined prior to the start of each experiment with the pH meter (Jenway model 3310, Jenway, Gransmore Green, UK). The carbonated solution was prepared by the investigators prior to each study, by adding 8 mg of CO₂ from a canister in 1 L of water in a commercially available soda maker (iSi, Siphon Soda-Seltzer maker©, iSi North America Inc., West Fairfield, NJ, USA), which holds the water at constant temperature (6°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. For non-carbonated solution intervention, we used bottled still water (Evian, Danone, France) which has a pH level of 7.2. The temperature in the glass containers used to hold the solutions during application, was constantly checked with lab thermometers throughout the study and were kept at 6°C. If the temperature changed during an on-going trial, another present member of the research team immediately changed the solution with one at the correct temperature and identical pH level.

Experimental Protocol:

Sixteen healthy participants (9 male, mean age 33.5 ± 4.3 , \pm SEM) were asked to attend the laboratory on three separate days with at least three days between each attendance to mitigate for any carry-over effect. Participants were asked to withhold from eating or drinking water or any beverage for at least 2 hours prior to each study. During their different visit days, they were presented with either one of the two different solutions to swallow or they were instructed to swallow their saliva, in a single-blinded randomized manner. At each visit, the participants sat comfortably in a reclining chair and pharyngeal sensory and tolerance levels measurements were obtained, as explained above. The cranial vertex was identified and marked on a surgical cap over their head. The cortical sites characterized as the sites evoking the largest pharyngeal/thenar responses in each hemisphere, were identified with mapping procedures using single TMS pulses delivered over the pharyngeal or thenar MC. Ten MEPs at motor threshold (MT)+20% intensity for each hemispheric site (stronger, weaker pharyngeal site and thenar representation) were recorded at baseline and at each of the post-intervention follow-up time-points. During recordings the participants were advised to withhold from any swallowing, coughing, talking, or moving their hands or arms.

Thereafter, the focal region of cortical suppression was created using 10 minutes of 1Hz rTMS over the stronger PMC site. They then received either the carbonated solution or non-carbonated solution (mineral water), or performed saliva swallows for 10 minutes as the “sensory bolus intervention”, according to the visit’s randomization. To complete the tasks, the liquid solutions were infused into the subject’s mouth with single use plastic syringes and manual injection of 3 mL boluses down a small plastic single-use tube. As each bolus was delivered into the mouth, the participants were asked to swallow on command to a visual cue which was a green circle appearing every 15s on a laptop monitor using a commercial presentation software (PowerPoint 2010; Microsoft Corporation, Redmond, WA, USA) placed in front of each volunteer. This was

performed for 10 mins, swallowing 3 mL boluses every 15s (or 40 swallows). For the saliva swallowing tasks comprised the same procedure using the same presentation software. All participants were instructed to keep the bolus in their mouth until instructed to swallow.

Following the interventions, the neurophysiological measurements were repeated immediately, then at 15, 30, 45 and 60 minutes. Figure 1 demonstrates the experimental protocol.

Fig 1. Protocol Schematic Representation

Around here.

Randomisation & Data Analysis:

Randomization was carried out using the block randomization option of the statistical software StatsDirect (Version 2.7, StatsDirect Ltd Cheshire, UK). All research data sets were anonymized.

Data analysis

The peak-to-peak amplitude of MEPs evoked by magnetic stimulation was used as a measure of motor cortex excitability. Signal software (CED, UK) was used to review individual MEPs in microvolts (μV) and to measure the amplitude of each trace. The individual average MEPs' amplitudes and latencies were calculated for each muscle group/cortical site and each time interval. In addition, the MEPs were then normalized to the baseline and are displayed as percentage change from baseline to minimize the inter-individual variability. Inter-individual factors such as age and sex were, therefore, equalized.

Statistical analysis

SPSS 25 (SPSS, Chicago, IL) was used for the statistical analysis of the normalized raw data. The data are expressed as mean (\pm SEM) unless stated otherwise. The normalized datasets, including each time-point except the baseline, were analyzed with repeated measures analysis of variance with factors: Intervention (carbonated, water and saliva), Time (immediately, 15, 30, 45 and 60 minutes) and Site (stronger and weaker pharyngeal and thenar representation). Post-hoc analysis using paired t-tests with Bonferroni correction was performed, if significant interactions between the factors were observed. The baseline sensory and tolerance thresholds across the three study arms were compared with non-parametric tests (Friedman's). In addition, AUC from percentage change analysis was employed to show the integrated magnitude of the responses of the participants, when ANOVA showed that time was not a significant factor, thus eliminating time-dependency effects. A $P < 0.05$ was taken as a measure of statistical significance. All data are presented as group mean \pm SEM, unless stated otherwise.

Results:

Studies were performed with no reported adverse incidents except for one subject suffering a possible provoked syncopal episode. This was fully investigated medically, and no clear cause or causal relationship to the study was found. The Health Research Authority and Hospital Ethics Committee were informed about the incident and all appropriate safety procedures were reviewed by the authorities. The subject remained well after the investigations were completed but, in view of the incident, this subject's (incomplete) data was removed from the analyzed dataset. Another subject's data were also excluded due to non-completion of the full protocol. Therefore, data reported here are based on the responses of fourteen completed datasets.

Baseline Sensory and Tolerance Thresholds and Cortical Motor Hotspots

The baseline sensory and tolerance thresholds across the three study arms were similar for each participant (Friedman test: sensory threshold: $\chi^2:0.3$, $P=0.862$, tolerance threshold $\chi^2:0.3$, $P=0.86$). The minimum (sensory) threshold ranged from 1.4 to 10.1 mA whereas the maximum (tolerance) threshold ranged from 4.0 to 31.1 mA.

Localisation of cortical representations PMEPs were recorded in all subjects. The stronger corticobulbar pharyngeal projection was found on the right hemisphere in 8 of 14 participants. The hemispheric site evoking the greatest pharyngeal response at the lowest thresholds (hotspot) was between 5.7 ± 0.8 cm (mean \pm SD) anterior to the vertex and 3.8 ± 0.7 cm lateral to the midline for the right hemisphere. In subjects who showed left hemispheric stronger pharyngeal representation, the optimal hotspot for stimulation was between 5.6 ± 1.0 cm anterior to the vertex and 3.9 ± 0.6 cm lateral to the midline.

The average TMS intensity to elicit PMEPs from the stronger PMC was $63.5 \pm 1.8\%$ (mean \pm SEM) whereas from the weaker hemisphere was $69.6 \pm 1.6\%$. The average TMS intensity to elicit TMEPs was $35.8 \pm 1.3\%$.

The baseline measurements of cortical excitability prior to the application of the different swallowed solutions were similar across all arms for each site: stronger and weaker pharyngeal representation and thenar cortical representation (Friedman's test, dominant cortical representation: $\chi^2: 3.9$, $P=0.145$, non-dominant cortical representation: $\chi^2: 1.3$, $P=0.52$, thenar cortical representation: $\chi^2: 1.9$, $P=0.395$).

MEP amplitudes

The group mean PMEPs and TMEPs amplitude percentage changes from the baseline across the different swallowing intervention at the different time-points are shown in Figs. 2A-C.

A three-way ANOVA used to analyze data with factors of Intervention (carbonated, water and saliva), Time (immediately, 15, 30, 45 and 60 minutes) and Site (stronger and weaker pharyngeal and thenar representation), showed a significant 3-way interaction ($F_{(1,13)}=5.2$, $P=0.039$).

Stronger pharyngeal projection:

A two-way ANOVA for the stronger corticobulbar pharyngeal projection showed a significant effect of Intervention on the percentage change of PMEPs for swallowing carbonated solution compared to still water and saliva ($F_{(1,13)}=7.5$, $P=0.017$) but no significant Time*Intervention interaction. There was, thus, an increase in the percentage change of the stronger projection following swallowing of carbonated solution for all the time points with the highest values from the baseline at 30 minutes ($80.3 \pm 39\%$) and 60 minutes ($74.6 \pm 27\%$), showing marked ability to reverse the anticipated inhibitory results of 1Hz rTMS (Fig. 3A). Given that the effects were not time-dependent, as shown with the ANOVA interaction above, Friedman's chi-square on AUCs of percentage change of the stronger pharyngeal representation across the 3 arms was performed and demonstrated that the distributions of the three interventions were different ($\chi^2:13.2$, $P=0.001$). Non-parametric Wilcoxon's test revealed a significant difference only between the carbonation and water-based protocols ($z=-3.05$, $P=0.002$) and between the carbonation and saliva-based protocols ($z=-2.6$, $P=0.008$).

Fig. 2A. Changes in corticopharyngeal MEPs following intervention on the suppressed hemispheric hotspot.

Around here

Weaker pharyngeal projection:

Figure 2B shows the changes in the PMC hotspot contralateral to the focal suppression following the swallowing intervention.

Fig. 2B. Changes in corticopharyngeal MEPs following intervention contralateral to the suppressed hemispheric hotspot.

Around here

There was a significant effect of Time*Intervention interaction for carbonated solution compared to saliva swallowing ($F_{(1,13)}=2.9$, $P=0.006$) and a significant effect of Intervention (carbonated solution) on the weaker pharyngeal representation compared to still water and saliva swallowing ($F_{(1,13)}=4.7$, $P=0.049$). During follow-up measurements on the weaker pharyngeal representation, it was evident that the carbonated solution swallowing protocol resulted in an increase in percentage change (the highest at immediately ($45.9 \pm 17.3\%$) and 15 minutes ($45.1 \pm 17.3\%$). As there was a Time interaction, paired t-tests were applied and a significant difference was observed between the carbonation and saliva-based protocol immediately post intervention ($t_{(13)}=2.5$, $P=0.024$), at 15 mins ($t_{(13)}=2.2$, $P=0.043$) and at 60 mins ($t_{(13)}=-2.7$, $P=0.017$) between carbonation and water-based protocol).

Fig. 2C. Changes in thenar MEPs following intervention.

Around here

Thenar projection

Figure 2C shows the percentage change in thenar site representation, acting as a control due to proximity to the pharyngeal projections. The thenar representation showed no significant Intervention*Time interaction ($F_{(1,13)}=0.29$, $P=0.6$) and a two-way ANOVA showed no effect of the different interventions on thenar cortical excitability ($F_{(1,13)}=0.37$, $P=0.551$). As there were no interactions, follow-up analyses were not undertaken.

MEP latencies

There was no significant difference in the MEP latencies across the different swallowing interventions and time-points (3-way ANOVA) within the pharyngeal representations and separately for thenar representation ($F_{(1,13)}=2.203$, $P=0.166$). Table 1 shows the mean MEP latencies \pm SEM (s) for the three hotspots across the different time-points.

Table 1. Response Latencies of the corticopharyngeal and thenar MEPs (in seconds, mean \pm SEM).

Around here

Discussion:

This study set out to investigate the extent to which a 10-minute interventional swallowing protocol with chemesthetic stimuli (carbonation) could interfere with or even reverse the effects of a focal suppression applied to pharyngeal MC following 1Hz rTMS, compared to water boluses and saliva swallowing sequences. It was evident that the carbonated swallowing interventional protocol had the capacity to modulate and seemingly reverse the inhibitory effects of 1Hz in the stronger (and

weaker) pharyngeal motor projections, compared to water-based and saliva-swallowing interventions. Our results merit further discussion.

Previously, we have reported that peripheral (pharyngeal electrical) stimulation [26] was sufficient to reverse the inhibitory effects of 1Hz rTMS through a cortically mediated effect. Interestingly, it has been demonstrated that in dysphagic patients following stroke, improvements in behavioral swallowing function and changes in cortical network were more strongly driven by peripheral (afferent) stimulation than brain TMS stimulation (excitatory rTMS) [30]. However, compared to other studies, here the methodology and characteristics of the intervention are quite different, given that the main medium is the act of swallowing itself. The subjects were asked to actively participate in their treatment regimen and exercise a timed-paced sensorimotor task of swallowing requiring the recognition of the stimuli, processing and planning of the motor action, the volitional coordination of respiration and swallowing, and triggering of the event to peripheral stimuli at a specific time [30]. This active form of participation in the intervention was coupled with the sensory stimuli of bolus' chemesthesis, namely carbonation in one arm versus no carbonated water bolus versus saliva (dry) swallows.

The delivery method of the intervention was kept identical as participants were instructed to perform the swallowing task with a specified time-interval between swallows and at a specific frequency with the identical cuing (visual) across all 3 arms. Thus, one would assume that comparing the effects of carbonated vs. water-based interventions, would inform us directly on the superiority of specific chemesthetic properties in reversing the focal suppression induced by the virtual lesion. For instance, the pH level was different between the carbonated and non-carbonated solutions. Yet, evidence from previous studies has shown that pH was unlikely to have been a factor. Indeed, we have compared the effects of carbonated water (pH level of 4.1) to non-carbonated, but citric acid based water (equi-pH level of 4.1) and shown that only carbonation was able to increase cortical excitability and show behavioural changes on a

swallowing reaction time task [6]. With regards to the level of the swallowing network where carbonation played a role, we speculate that the superiority of carbonation occurred mechanistically at the cortical level, since the MEP latencies of both pharyngeal and thenar representations and the cortical excitability of the thenar representation (control) remained unchanged across the different paradigms.

Of interest, in all 3 arms, changes following the different swallowing protocols were similar in both the unsuppressed and suppressed pharyngeal cortical representation, albeit to a smaller degree in the unsuppressed hemisphere with the carbonation-based protocol showing superiority compared to the other protocols at specific time-points. This is interesting, given that we know from previous studies that the focal suppression to the stronger pharyngeal representation has not been shown to impact the contralateral site after 1Hz rTMS [4,27,31]. Here, we observed an increase in the cortical excitability following carbonated bolus swallowing in the unsuppressed hemispheric site at nearly all the study time-points, which we assume is mainly driven by the afferent stimulation of the treatment per se. This, of course, is in keeping with the knowledge that swallowing neural network is regulated by bilateral non-competitive interhemispheric cortical processes [29,32,33] and therefore this bilateral increase in cortical excitability following afferent stimulation might have been anticipated. In keeping with previous studies with carbonation vs. water vs. citric acid boluses [6], there is marked increase in cortical excitability in both hemispheres following the introduction of carbonated boluses. This has also been observed in another recent study [7] where carbonation showed a short-lived but significant increase in cortical excitability immediately after the delivery of carbonated boluses.

Our data support the notion that carbonation affects swallowing through a centrally mediated mechanism that is likely to be driven by afferent stimulation. There are already findings that show sour cells on the tongue provide the cellular sensors for carbonation, allowing the stimulation of the taste system [34]. The conversion of CO₂ to carbonic acid in carbonated water, a reaction

catalyzed by carbonic anhydrase, leads to activation of lingual nociceptors which excite trigeminal neurons, involved in signalling oral irritation to higher centres [15,35]. Therefore, carbonation probably produces its effects through changes in higher centres involved in swallowing and taste. This may explain why the carbonated bolus swallowing paradigm showed greater capacity to reverse the focal suppression in the stronger, albeit suppressed, pharyngeal representation. By contrast, still water and saliva swallows alone may be ineffective as a medium in facilitating sustained changes to the swallowing system as far as the reversal of the focal suppression is concerned. Those two boluses are mainly used in clinical settings by therapists to support their rehabilitation program, yet our findings do not strongly support their clinical utility if used alone. Previously, Fraser et al [36] have observed that following a swallowing regime of 10 minutes of volitional water swallowing of 5ml boluses every 5s (in total 120 swallows) with no preceding focal suppression resulted in short-term changes in pharyngeal and esophageal MEP amplitudes. Maximal facilitation occurred mainly in the period immediately after swallowing before returning to baseline by 15 minutes. Although the total number of the swallows in Fraser et al protocol [36] was larger compared to the total of 40 swallows we have used here, it seems that our results follow the same trend with the change in MEP amplitude being larger immediately after the end of the intervention. This could infer that swallowing of water or saliva swallows is not effective enough to reverse the cortical suppression and it could pose important clinical implications when treating dysphagic stroke patients. Further work on dysphagic stroke patients is needed, to better understand how to more effectively apply such “tastant” stimuli to the swallowing network and improve swallowing function.

Limitations to this study include the lack of a no-swallowing paradigm arm, and hence not quantifying the effects of focal suppression alone at an individual level. However, here, the main research question was which swallowing paradigm would present superiority to reverse the suppression and the effects of the 1Hz rTMS on swallowing neural network are already well-

established. One further limitation is that our findings are only generalizable to young healthy adults. Future studies should include participants of different age groups. With respect to our current study, we should point out that the raw data baseline measurements (ranging from pharyngeal sensory threshold to MEP amplitude) were similar to other studies performed previously [6-7] on estimating the excitation of the corticobulbar tracts after an intervention. This adds creditability to arguments including comparisons to other studies. Lastly, solutions' temperature used and tested in this protocol were not similar across all three arms. Both for the water and carbonation boluses, the temperature was similarly low. However, for the saliva arm, the level would have been at body temperature. Thermal stimulation from the cold bolus could have affected the results, given that previous studies have shown that cold boluses can change swallowing physiology when taken in larger quantities [2]. However, the fact that both the water and saliva swallowing arms showed similar profiles of effects post the virtual lesion suggests that the effect of temperature in this model was minimal.

In conclusion, carbonated boluses delivered in an interventional protocol with specified parameters had the capacity to reverse the focal suppression with 1Hz rTMS. Our results support the notion that carbonation may be a helpful adjunct in the rehabilitation of dysphagia after stroke and serves as a prelude to the application of swallowing interventions with chemesthetic stimuli in dysphagic populations.

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Disclosures: Nothing to disclose

Author contributions

Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by EM and SH. The first draft of the manuscript was written by EM and both authors commented on previous versions of the manuscript. Both authors read and approved the final manuscript.

Funding

Funding: The authors did not receive support from any organization for the submitted work. No funding was received to assist with the preparation of this manuscript.

Acknowledgements

The authors gratefully acknowledge the support of the following for the completion of the project: Omsaad Elshukri, Dipesh Vasant, Mary Louise Harris and Hannah Mentz. The authors are also grateful to the volunteers for their time and participation in this project.

Figures Legends:

Fig. 1: Schematic demonstrating the procedures over time for the protocol. TMS: Transcranial Magnetic Stimulation.

Fig. 2A. Mean percentage change in PMEP amplitudes following carbonated, still water and saliva intervention. PMEPs are plotted as mean data \pm SEM. Carbonated solution intervention showed a dramatic increase in cortical excitability and reversal of focal suppression across Time, not seen with water or saliva swallowing interventions.

Fig. 2B. Mean percentage change in PMEP amplitudes following carbonated, still water and saliva swallowing intervention. Carbonated solution intervention showed an increase in cortical excitability across all time points compared to water and saliva.

Fig. 2C: Mean percentage change in TMEP amplitudes following carbonated, still water and saliva swallowing intervention. Carbonated solution intervention showed an increase in cortical excitability across all time points being significantly more compared to water and saliva.

Table 1. Tabulation of the PMEPs and TMEPs across the three different study arms (in s).

Fig. 1: Protocol Schematic Representation

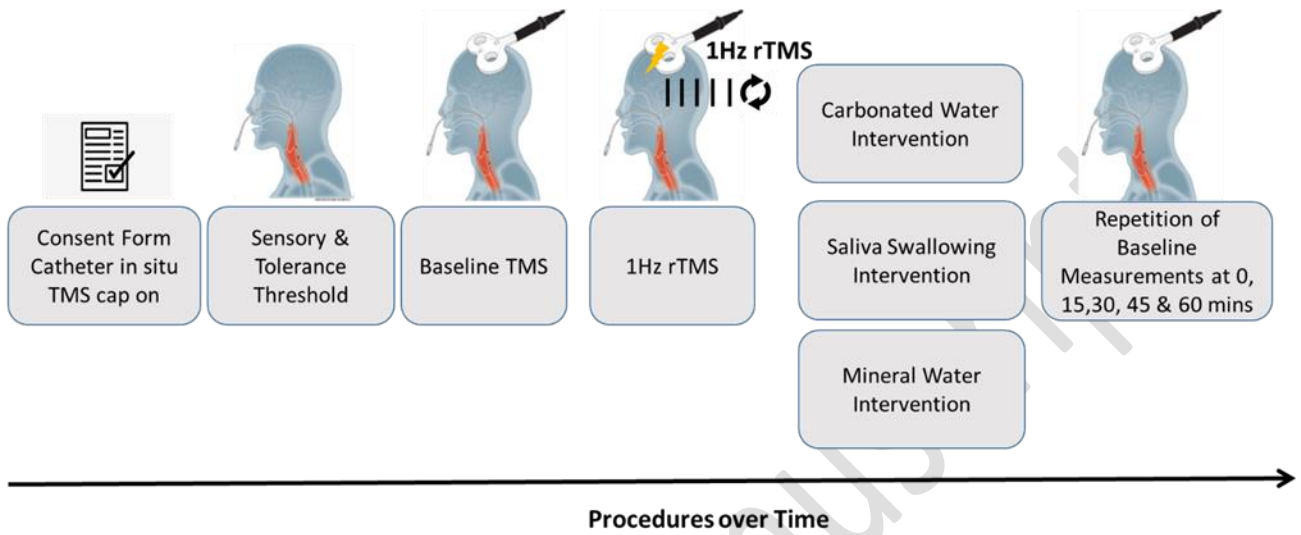


Fig. 2A: Changes in corticopharyngeal MEPs following intervention on the suppressed hemispheric hotspot.

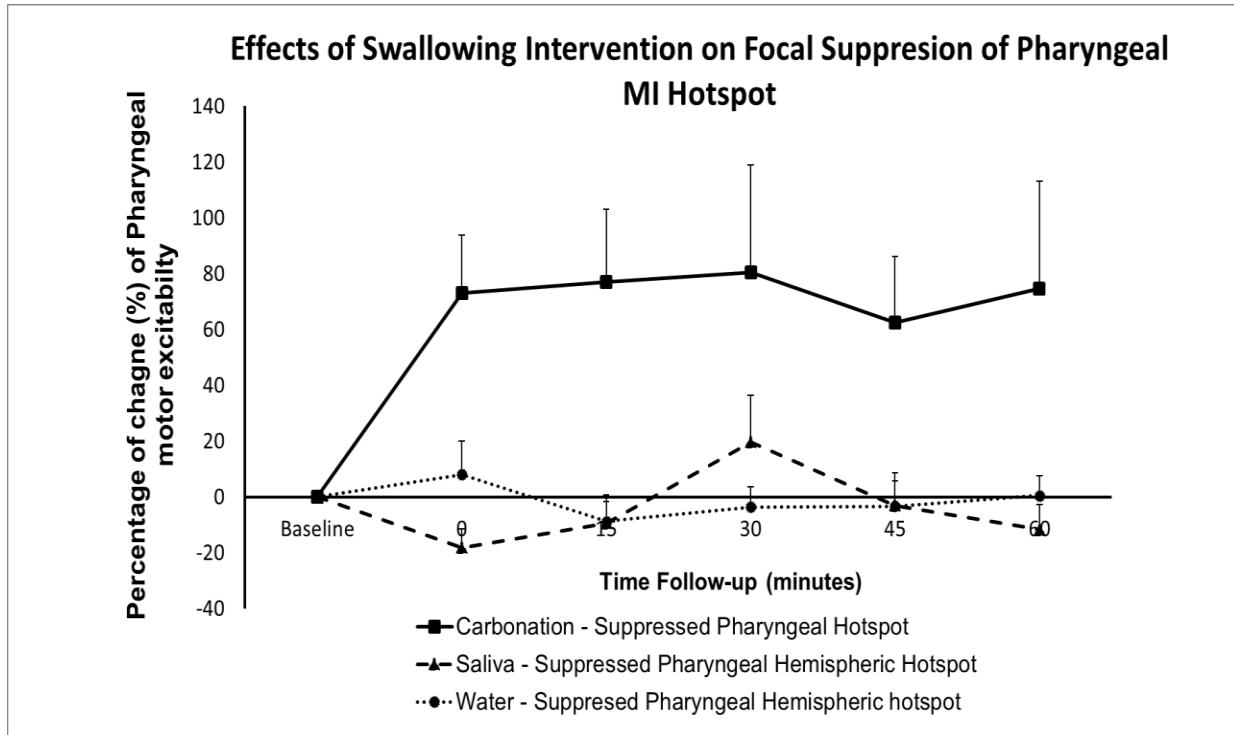


Fig. 2B: Changes in corticopharyngeal MEPs following intervention contralateral to the suppressed hemispheric hotspot.

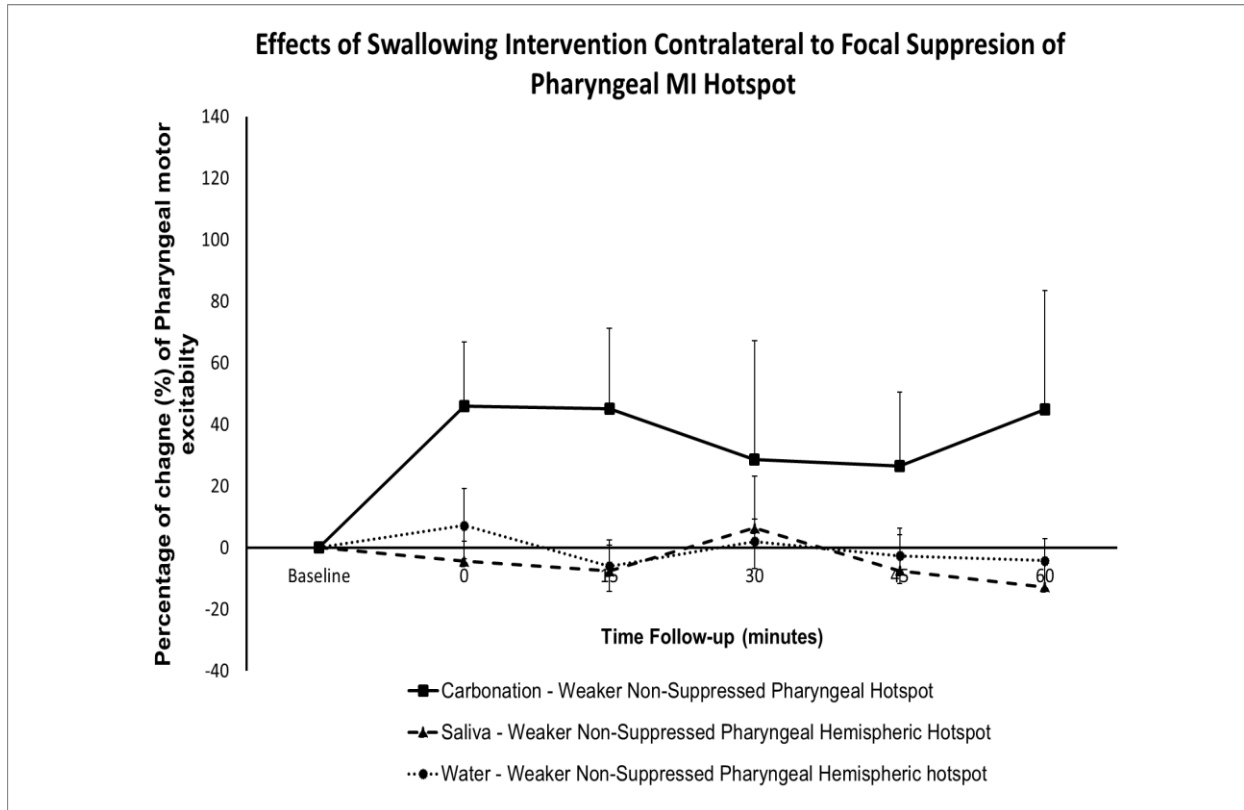


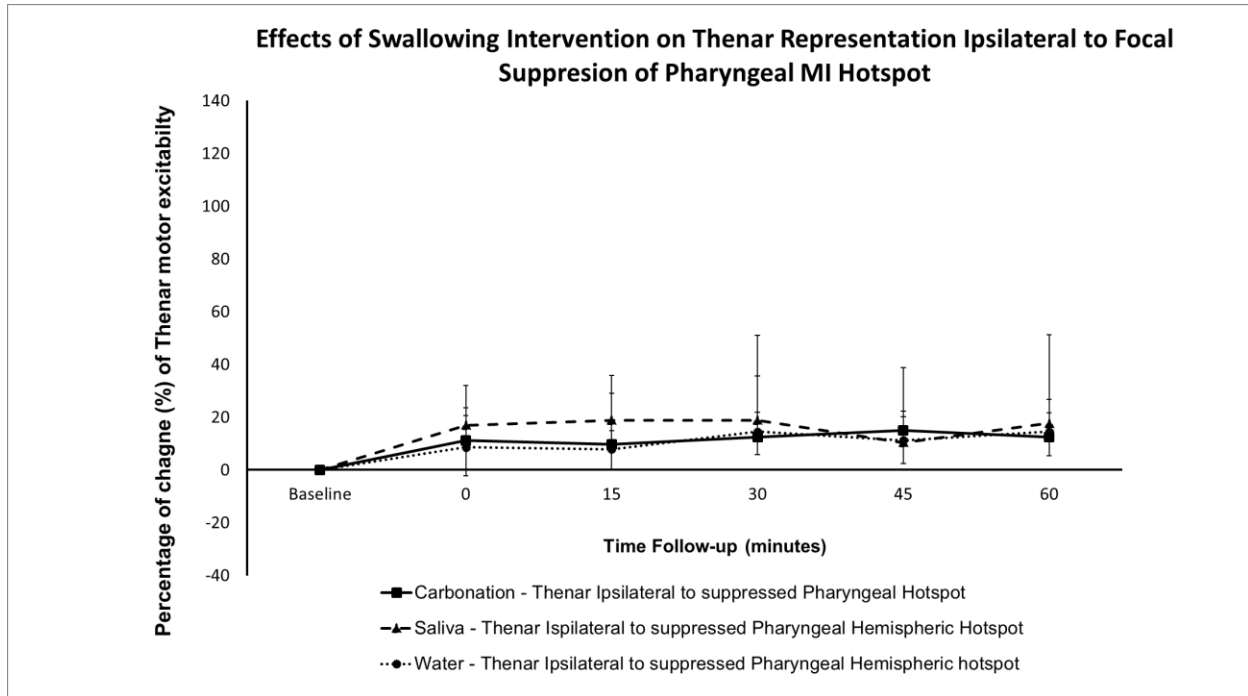
Fig. 2C: Changes in thenar MEPs following intervention.

Table 1. Response Latencies of the corticopharyngeal and thenar MEPs (in seconds, mean \pm SEM).

Site	Baseline	0mins	15mins	30mins	45 mins	60 mins
Non-Carbonated Bolus						
Strong Representation	0.084 \pm 0.021	0.085 \pm 0.028	0.083 \pm 0.019	0.086 \pm 0.021	0.083 \pm 0.015	0.086 \pm 0.013
Weaker Representation	0.085 \pm 0.016	0.088 \pm 0.015	0.082 \pm 0.015	0.091 \pm 0.010	0.090 \pm 0.015	0.092 \pm 0.015
Thenar Representation	0.021 \pm 0.010	0.021 \pm 0.011	0.021 \pm 0.012	0.022 \pm 0.013	0.022 \pm 0.015	0.022 \pm 0.012
Carbonated Bolus						
Strong Representation	0.086 \pm 0.019	0.088 \pm 0.020	0.085 \pm 0.021	0.088 \pm 0.022	0.089 \pm 0.020	0.088 \pm 0.016
Weaker Representation	0.088 \pm 0.021	0.084 \pm 0.020	0.086 \pm 0.021	0.087 \pm 0.019	0.085 \pm 0.018	0.088 \pm 0.015
Thenar Representation	0.021 \pm 0.011	0.022 \pm 0.012	0.021 \pm 0.013	0.022 \pm 0.011	0.019 \pm 0.013	0.020 \pm 0.011
Saliva Swallows						
Strong Representation	0.090 \pm 0.019	0.087 \pm 0.021	0.090 \pm 0.020	0.088 \pm 0.022	0.093 \pm 0.021	0.091 \pm 0.015
Weaker Representation	0.087 \pm 0.025	0.090 \pm 0.020	0.090 \pm 0.021	0.085 \pm 0.020	0.093 \pm 0.019	0.085 \pm 0.020
Thenar Representation	0.021 \pm 0.010	0.024 \pm 0.011	0.022 \pm 0.012	0.021 \pm 0.015	0.022 \pm 0.016	0.021 \pm 0.017