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Exploring parameters of gamma transcranial alternating current stimulation (tACS) and full-spectrum transcranial random noise stimulation (tRNS) on human pharyngeal cortical excitability

Mengqing Zhang[1,2] | Ivy Cheng[2] | Ayodele Sasegbon[2] | Zulin Dou[1] | Shaheen Hamdy[2]

[1]Department of Rehabilitation Medicine, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

[2]Division of Diabetes, Endocrinology and Gastroenterology, Faculty of Biology, Medicine and Health, Centre for Gastrointestinal Sciences, Clinical Sciences Building, Salford Royal NHS Foundation Trust, School of Medical Sciences, The University of Manchester, Manchester, UK

Correspondence
Shaheen Hamdy, Division of Diabetes, Endocrinology and Gastroenterology, Faculty of Biology, Medicine and Health, Centre for Gastrointestinal Sciences, Clinical Sciences Building, Salford Royal NHS Foundation Trust, School of Medical Sciences, The University of Manchester, Salford, M6 8HD, UK.
Email: shaheen.hamdy@manchester.ac.uk

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Abstract

Background: Transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) have been shown to have physiological and functional effects on brain excitability and motor behavior. Yet, little is known about their effects in the swallowing system.

Aim: To examine the effects and optimal stimulation parameters of tACS and tRNS for modulating excitability of human pharyngeal motor cortex.

Methods: 10 Hz (alpha), 20 Hz (beta), 70 Hz (gamma) tACS, 0.1–640 Hz (full-spectrum) tRNS, and sham were applied over pharyngeal motor cortices at 1.5 mA current intensity for 10 min in 15 healthy participants. Pharyngeal motor–evoked and thenar motor–evoked potentials (PMEPs and TMEPs) were assessed before and up to 2 h after stimulation with single-pulse transcranial magnetic stimulation. Averaged MEP amplitude and latency changes were analyzed using repeated measures ANOVA (rmANOVA).

Key Results: Two-way rmANOVA across all active interventions demonstrated a significant MEP interaction both in the stimulated pharyngeal cortex ($F(4, 56) = 1.731$, $p = 0.038$) and in the ipsilateral thenar cortex ($F(4, 56) = 1.506$, $p = 0.048$). Compared to sham, subsequent *post hoc* tests showed site-specific and sustained (60–120 min) increases in PMEPs with gamma tACS and tRNS ($p = 0.005$, $p = 0.027$, respectively) and for TMEPs with beta tACS ($p = 0.006$).

Conclusions and Inferences: Our findings suggest that the effects of tACS and tRNS are frequency-dependent and cortical (representation) site-specific with both gamma tACS and full-spectrum tRNS enhancing human pharyngeal cortical excitability. These techniques hold promise as potential treatments for neurological dysphagia.

KEYWORDS
gamma frequency, pharyngeal cortical excitability, swallowing, transcranial alternating current stimulation, transcranial random noise stimulation

1 | INTRODUCTION

Transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) are two novel, non-invasive brain stimulation (NIBS) techniques that deliver low-intensity sinusoidal alternating current (AC) continuously over the cerebral cortex.[1] Both techniques at low stimulation intensities are safe and well-tolerated in healthy adults and patients[2] and directly alter excitability within the brain for periods outlasting the duration of stimulation.[2] When used to modulate excitability within the primary motor cortex (M1), tACS and tRNS have been shown to have physiological and functional effects on both hand motor excitability and behavior.[3-5] Compared to transcranial direct current stimulation (tDCS), tACS and tRNS have similar effects on cortical excitability but appear to produce less unpleasant sensations when applied to the scalp,[5-7] thus conferring a potential advantage to clinical utilization.

Recent studies suggest that brain stimulation leads to swallowing recovery.[8,9] As we know, swallowing is a complex and well-coordinated process which is associated with activation of several areas of the central nervous system (CNS) for its safe deployment. Moreover, swallowing problems (dysphagia) commonly occur following neurological disorders such as stroke and/or among the elderly population.[10] Complications include pneumonia, dehydration, malnutrition, or even increased mortality.[10] Although growing numbers of studies have demonstrated that both repetitive transcranial magnetic stimulation (rTMS)[8] and tDCS[9] can be used to modulate both excitability of pharyngeal motor cortex and swallowing behavior, there remains limited evidence for the efficacy of such treatments on health measures such as pneumonia and mortality. Therefore, any new therapy that has the potential to make a significant difference to the quality of life for these patients would be welcomed.

Transcranial alternating current stimulation allows manipulation of neural oscillations in the cortical region being stimulated.[1,11] Brainwaves or neural oscillations (frequency bands: delta 1–4 Hz, theta 4–7 Hz, alpha 8–12 Hz, beta 13–30 Hz, gamma 30–200 Hz) have been shown to play important roles in motor, perceptual, and cognitive functions.[12,13] For instance, oscillatory activity at the beta range might mediate the control of more complex movements in M1,[14] whereas gamma oscillations were found to be stronger for larger movements.[15] By applying AC through two electrodes attached to a subject's scalp, it is possible to entrain the intrinsic oscillation of the cortex directly under one electrode to a specific frequency.[16] For the motor cortex, alpha, beta, and gamma frequencies are the main oscillations.[17] Previous studies have demonstrated that applying tACS over hand M1 resulted in measurable changes of hand movement velocity and force at beta and gamma band frequencies.[18] TACS at 80 Hz over M1 and cranial vertex was also reported to improve the performance of a visuomotor tracking task.[18] Furthermore, multiple sessions of tACS can be used to induce neuroplastic changes that outlast the duration of stimulation.[19] For example, Kasten et al.[20] found the tACS physiological after-effect could last up to 70 min.

By comparison, tRNS is a variant form of tACS where AC is applied while both intensity and frequency of the current vary in a randomized manner. Terney et al.[5] demonstrated that tRNS applied over M1 is capable of changing both cortical excitability and behavior in healthy participants. In most of the studies using tRNS, a frequency spectrum between 0.1 Hz and 640 Hz (full spectrum) or 101–640 Hz (high-frequency stimulation) were used.[7,21] Interestingly, the after-effect of tRNS was intensity-dependent. High-intensity stimulation, for example, 1 mA, resulted in facilitatory after-effects of up to 1.5 h in M1.[22]

KEY POINTS

- Transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) are novel transcranial electrical stimulation technologies for non-invasive brain stimulation.
- Both gamma tACS and full-spectrum tRNS can enhance human pharyngeal cortical excitability.
- These techniques hold promise as potential treatments for neurological dysphagia.

Based on these somatic studies, we hypothesized that tACS at alpha, beta, and gamma frequencies and at full-spectrum frequency tRNS would selectively modulate pharyngeal motor cortex excitability and induce sustained after effects. Thus, our aims were to examine the effects of different frequencies of tACS (at 10 Hz, 20 Hz, 70 Hz) and tRNS (at 0.1–640 Hz) to determine the optimal stimulation parameters for modulating the excitability of the pharyngeal motor cortex, as a prelude to studying the therapeutic effects of tACS or tRNS in patients with (neurological) dysphagia.

2 | METHODS

2.1 | Subjects

Following estimates of pharyngeal cortex effects with tDCS,[23] a sample size of 12 was calculated to achieve a power of 80% and statistical significance of 5% with G*Power Statistics (version 3.1). We therefore chose to recruit a minimum of 14 subjects to allow for dropouts and incomplete data acquisition, based on the results of previous studies within our department.[10,23] Seventeen healthy volunteers were recruited and 15 (six males, age range 18–50 years, mean (±SEM) 24 ± 8 years) completed the entire study. Fourteen were right-handed. All subjects were in good health and able to give written, informed consent. Exclusion criteria, based on a standard pre-screening questionnaire,[2] included the following: history of bipolar disorder, depression, epilepsy, cardiac pacemaker, implanted metal, skin problems, brain surgery, head trauma, swallowing problems, and use of medication which acts on the central nervous system or pregnancy. The study was conducted in accordance with the World Medical Association Declaration of Helsinki. Ethical approval was obtained from local Research Ethics Committee of the

University of Manchester (Approval No: 2019-5932-10164; Date: 17 April 2019), United Kingdom. Written informed consent was obtained from each participant prior to the experiment.

2.2 | Pharyngeal motor–evoked and thenar motor–evoked potentials measurements

Pharyngeal motor–evoked potentials (PMEPs) were recorded from a pair of bipolar platinum ring electrodes built into a 3.2 mm diameter intraluminal catheter (Gaeltec Ltd,). Participants were asked to swallow the catheter, passed either transnasally or transorally according to their preference such that the electrodes were in contact with the pharyngeal musculature, approximately 2 cm above the upper esophageal sphincter. An earth electrode (H69P, Tyco Healthcare) was connected to a skin electrode placed over the upper portion of one of the sternocleidomastoid muscles in the neck. As a control, thenar motor–evoked potentials (TMEPs) from the abductor pollicis brevis (APB) muscle were recorded using two surface electrodes, sited 1 cm apart on the thenar eminence muscle, contralateral to the stimulated pharyngeal motor cortex (see below). An additional earth electrode was connected to a skin electrode positioned over a bony prominence on the wrist. All the electrodes were connected via a preamplifier (CED 1902; Cambridge Electronic Design) with high- and low-pass filter settings of 200 Hz and 2 kHz. Response signals were collected through a laboratory interface (CED micro 1401) at a sampling rate of 5 kHz and recorded using software Signal Application Program v.4.11 (Cambridge Electronic Design Ltd,) running on a personal computer. To remove any unwanted electrical interference, the signals were additionally processed through a 50/60 Hz noise eliminator ("HumBug"; Quest Scientific). These techniques for recording PMEPs and TMEPs are well established and have shown stability of measurements in previous studies.[8,9,23]

2.3 | Single-pulse transcranial magnetic stimulation

Single-pulse TMS was delivered over the regions of interest on the scalp using a figure-of-eight coil with a 70 mm outer diameter. The coil was connected to a stimulator (Magstim 200; The Magstim Company) with a maximum output of 2.2 Tesla. The coil handle was held in an anteroposterior position at an angle of 45 degree tangential to the midsagittal line of the scalp as previously described.[24]

2.4 | Sensory side effects questionnaire

During the experimental procedures, non-serious adverse reactions were recorded by a researcher on a standard questionnaire of sensations ([Appendix S1],[2] which contains detailed questions regarding a list of known adverse events [eg, phosphenes [illusory flash-like visual percepts], burning sensation, pain, itching, and headache]).

2.5 | Transcranial alternating current stimulation and transcranial random noise stimulation

TACS and tRNS were delivered through a CE (European Conformity, which indicates conformity with health, safety, and environmental protection standards for products sold within the European Economic Area) marked battery-driven constant current stimulator (DC Stimulator Plus, NeuroConn, Ilmenau, Germany) connected to a pair of rectangular electrodes (5 × 7 cm, current density 0.043 mA/cm^2). The center of stimulating electrode was positioned on the scalp over the "pharyngeal" area of the motor cortex producing the largest MEPs and the reference electrode overlying the contralateral supraorbital ridge to minimize any unintended effect of the other cortex.[25] To ensure optimal contact with the scalp, a saline-soaked sponge was placed beneath both electrodes, and the electrodes were then held in place by adjustable rubber straps. Since initial studies of the M1 hand area applied different tACS frequency paradigms[18-22] that demonstrated significant enhancement in cortical excitability, our initial investigation duplicated these parameters in the pharyngeal motor system. Moreover, the intensity and duration of stimulation in this experiment were identical to the parameters used in a previous tDCS dose-response study at 1.5 mA for 10 min where an increase in pharyngeal cortical excitability was reported.[23] For active intervention, the current was slowly ramped up to 1.5 mA (peak-to-peak) over 10 s and maintained for 10 min, before being slowly ramped down over 10 s.[7,23] For the sham condition, the current was turned off after 10 s of 20 Hz-tACS stimulation with the electrodes being left in place for a further 10 min, thus producing a similar sensation as the active treatment but without significantly stimulating the cortex.[26] Impedance was monitored while stimulation and kept below 10 kΩ for all studies. All tACS and tRNS applications in this study complied with published safety guidelines.[2]

2.6 | Experimental protocol

Participants (n = 17) were seated in a comfortable chair with armrests and wore disposable surgical caps for the marking of stimulation "hot-spots." The pharyngeal catheter was then sited, guided by online raw EMG analysis to determine where the upper esophageal sphincter was located, and electrodes retracted aborally 2 cm so they sat in the mid-pharynx. Thenar electrodes were also attached to the participant. Motor hot spots and thresholds for pharynx and hand were determined per the TMS methods outlined above. The hot spot is defined as the location which has the lowest resting motor threshold (rMT) with the largest MEP[27] from the target muscle elicited by single-pulse TMS. RMT is defined as the minimum stimulation intensity that can produce MEPs of at least 20 μV for the pharynx and 50 μV for the thenar muscle in 50% of 10 consecutive trials in the resting state.[27] The three hot spots were determined over both hemispheres for pharyngeal cortex and over the hemisphere with the largest PMEP for the hand motor cortex. The

hemisphere evoking the largest PMEPs was defined as the tRNS- or tACS-stimulated (dominant) hemisphere. Baseline responses were recorded as 2 sets of 10 single-pulse TMS stimuli over both hemispheres for the pharynx and over the stimulated hemisphere for thenar muscles, applied to each site at rMT +20% stimulator output. An interval of five seconds was left in between each TMS stimulus during MEP assessment. Following baseline measurements, each participant received all of the different frequency paradigms of tACS (10 Hz, 20 Hz, and 70 Hz) and tRNS (0.1–640 Hz) and sham over different days (see below), with one stimulation/sham paradigm being delivered per visit. Volunteers received the active tACS and tRNS interventions over pharyngeal motor cortex with the lowest rMT at 1.5 mA current intensity for 10 min; sham stimulation was given as described in the methods above. Cortical excitability of each hot spot was then assessed by a set of 10 single-pulse TMS per hot spot at rMT +20% stimulator output, immediately and then repeated every 15 min for 2 h post-intervention. Each study took place on separate days, at least 4 days apart, and the order of the studies was pseudorandomized for each participant using a random number generator. The interventions were given by an independent researcher and single-blinded such that participants were blinded to group allocation. The primary study endpoints were the percentage changes of MEPs amplitudes and latencies (normalized to baseline) over time.

2.7 | Data analysis

The amplitude was defined as the maximum peak-to-peak voltage of each MEP, and the latency was the duration measured in milliseconds from time zero (time of TMS stimulation) to the onset of each MEP signal. The amplitudes and latencies of individual PMEPs and TMEPs were determined from each group of 10 EMG traces (for each site and intensity) and then averaged. In order to minimize variability, these data were then normalized to baseline (taken as the average of 2 × 10 pulses for each site) and expressed in the results as a percentage change from baseline.

2.8 | Statistical methods

All statistical analyses were performed using SPSS 23 (SPSS Inc,). Changes in excitability over time between the different groups and sham were compared using a general linear model two-way repeated measures analysis of variance (two-way rmANOVA). When significant effects were present, these were followed up with post hoc analysis including adjustment for multiple comparisons (Bonferroni correction) to explore the strength of the main effects. Non-sphericity was corrected using Greenhouse-Geisser where necessary. The above analyses were performed for the MEP amplitude and latency data using the percentage changes from baseline which displayed a normal distribution. Statistical significance was taken as $p < 0.05$.

3 | RESULTS

Two participants (of the initial 17 recruited) did not complete the experiment due to pharyngeal catheter intolerance or study withdrawal. Hence, 15 healthy volunteers completed the full protocol; TMS, tACS, and tRNS were tolerated well without any serious adverse events.

3.1 | Sensations questionnaire

The main participant side effects were phosphenes and mild unpleasant scalp sensations (eg, itching and tingling). Scalp sensations and phosphenes were most prominent with tACS at frequencies of 10 and 20 Hz and diminished at the higher frequency (70 Hz). Phosphenes were reported by all fifteen subjects during 10 Hz and 20 Hz tACS interventions (Figure 1). It was rated as weaker and was less frequently reported at 70 Hz tACS and with tRNS. Reported scalp sensations were described in up to 60% of participants across all the interventions. Of relevance, in the sham group, 80% of subjects reported phosphenes and 40% had scalp sensations. Evaluation of the blinding procedure showed that no subjects could identify whether they were receiving active or sham stimulation.

3.2 | Cortical hot spot mapping and resting motor thresholds

Average pharyngeal motor threshold to TMS was 67% (±8%) of stimulator output over the stimulated (dominant) hemisphere (range 53–82%) and 75% (±9%) for the unstimulated (non-dominant) hemisphere (range 56–90%). Mean rMT for thenar motor cortex

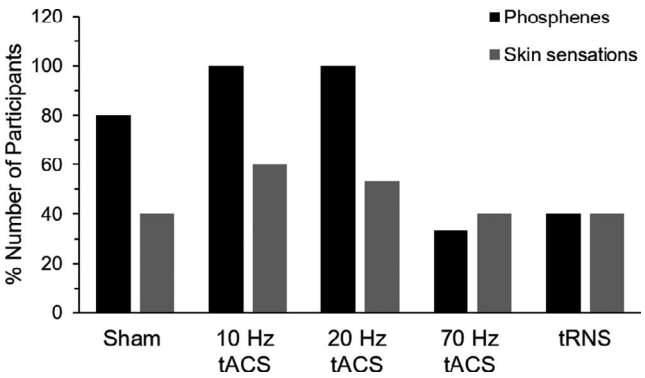

FIGURE 1 Sensory side effects of tACS and tRNS elicited by different stimulation set-ups; phosphenes were reported by all subjects at both 10 Hz and 20 Hz tACS condition but diminished with 70 Hz tACS and tRNS. Reports of scalp sensations were ranged from 40% to 60% among all the set-ups. Sham group also reported effects, with 80% of subjects reported phosphenes and 40% scalp sensations

was 44% (±10%) stimulator output (range 32–65%). The average distance from the cranial vertex to motor hot spots was: right pharyngeal hemisphere 4.0 ± 0.6 cm lateral and 3.2 ± 0.8 cm anterior and the left pharyngeal hemisphere 4.1 ± 0.9 cm lateral and 3.1 ± 0.4 cm anterior; and right thenar (left M1) 6.2 ± 0.7 cm lateral and 1.3 ± 0.8 cm anterior and left thenar (right M1) 5.0 ± 0.6 cm lateral and 1.9 ± 0.9 cm anterior. Baseline MEP amplitudes and latencies for all interventions are shown in Table 1. Figure 2 shows representative PMEPs and TMEPs data from one participant during their evaluation.

3.3 | The effects of tACS and tRNS on cortico-pharyngeal motor excitability

The mean response amplitudes at baseline and each time point for PMEPs in the stimulated hemisphere following tACS and tRNS are shown in Figure 3A. Compared with sham, both 70 Hz tACS and full-spectrum tRNS at 1.5 mA for 10 min produced increases in cortical excitability for the pharynx in the conditioned (stimulated) hemisphere [$F(1,14) = 9.065$, $p = 0.005$ and $F(1,14) = 5.394$, $p = 0.027$, respectively], with increases in PMEP amplitude of up to +77 ± 24% and +59 ± 30%, respectively. By contrast, 10 Hz tACS appeared to slightly suppress pharyngeal cortical excitability, with a trend to decreased PMEP amplitudes of −30 ± 7%. Moreover, 20 Hz tACS elicited no obvious changes in the amplitude of PMEPs. Two-way repeated measures ANOVA on normalized MEP data with factors of interventions (10 Hz tACS, 20 Hz tACS, 70 Hz tACS, tRNS, and sham), and time (immediately and every 15 min post-intervention) revealed a significant stimulation × time interaction ($F(4,56) = 1.731$, $p = 0.038$) only in the first 60 min after stimulation. *Post hoc* analysis revealed significant increases in PMEP amplitudes for overall 70 Hz tACS ($p = 0.005$) (at immediately post-stimulation ($p = 0.019$), at 45 ($p = 0.004$) and 60 min ($p = 0.011$)) and overall tRNS conditions ($p = 0.027$) (at 15 ($p = 0.023$) and 45 min ($p = 0.025$), compared to sham. PMEP amplitudes over the unstimulated hemisphere ($F(4,56) = 0.896$, $p > 0.05$) and latencies of all MEPs did not reveal significant intervention interactions (Figures 3 and 4).

3.4 | The effects of tACS and tRNS on cortico-thenar motor excitability

By comparison, two-way repeated measures ANOVA revealed that there was also a significant stimulation × time interaction ($F(4, 56) = 1.506$, $p = 0.048$) on TMEP amplitudes (Figure 3C). *Post hoc* tests revealed a significant increase in TMEPs for the 20 Hz tACS condition immediately after stimulation, sustained to 120 min, compared to sham ($p = 0.006$). No significant effects on TMEPs were found after tRNS and 10 Hz or 70 Hz tACS (Figure 3C). As with PMEPs, there was no interaction for the thenar latencies with any intervention (Figure 4).

4 | DISCUSSION

The present study aimed to examine the effects (and side effects) of 10, 20, 70 Hz tACS, and tRNS on excitability of the pharyngeal motor cortex in humans. We found that gamma tACS and full-spectrum tRNS increased the excitability of pharyngeal motor cortex while beta tACS only induced excitatory effects in ipsilateral hand motor cortex. While we did not use neuro-navigated TMS for cortical motor mapping, the latencies were stable from both hand and pharynx hot spots which suggest stability of the TMS coil location. Moreover, these facilitated changes were sustained for 60 to 120 min following stimulation. The differences in NIBS after-affects between the 2 motor systems (hand/thenar, pharynx) indicate different neuroplasticity mechanisms that appear to depend on the frequency and target site of alternating current electricity. These findings are of interest and therefore merit further discussion.

4.1 | Neurophysiological effects of tACS and tRNS on pharyngeal motor cortex

In the present study, both gamma band tACS and full-spectrum tRNS provoked excitatory effects in the stimulated pharyngeal motor cortex. These findings suggest that the AC effects on swallowing cortex are frequency-dependent, similar to the effects in other

TABLE 1 Mean baseline (±SD) motor-evoked potential (MEP) amplitudes and latencies for all interventions

	10 Hz tACS	20 Hz tACS	70 Hz tACS	tRNS	sham
Amplitude (μV)					
Stimulated pharyngeal	94.3 ± 53.5	88.7 ± 36.7	89.1 ± 46.6	83.6 ± 40.7	107.0 ± 64.3
Unstimulated pharyngeal	93.1 ± 72.1	87.4 ± 56.8	97.5 ± 41.5	90.8 ± 52.6	95.9 ± 59.2
Thenar	979.1 ± 873.6	969.0 ± 586.3	973.7 ± 770.4	934.1 ± 681.6	905.5 ± 455.9
Latency (ms)					
Stimulated pharyngeal	8.8 ± 1.3	8.3 ± 0.8	8.4 ± 1.1	8.7 ± 0.9	8.8 ± 1.1
Unstimulated pharyngeal	8.7 ± 0.9	8.3 ± 0.7	8.4 ± 0.8	8.5 ± 0.8	8.7 ± 1.1
Thenar	20.9 ± 1.7	21.0 ± 1.6	21.1 ± 1.5	21.1 ± 1.7	20.7 ± 1.8

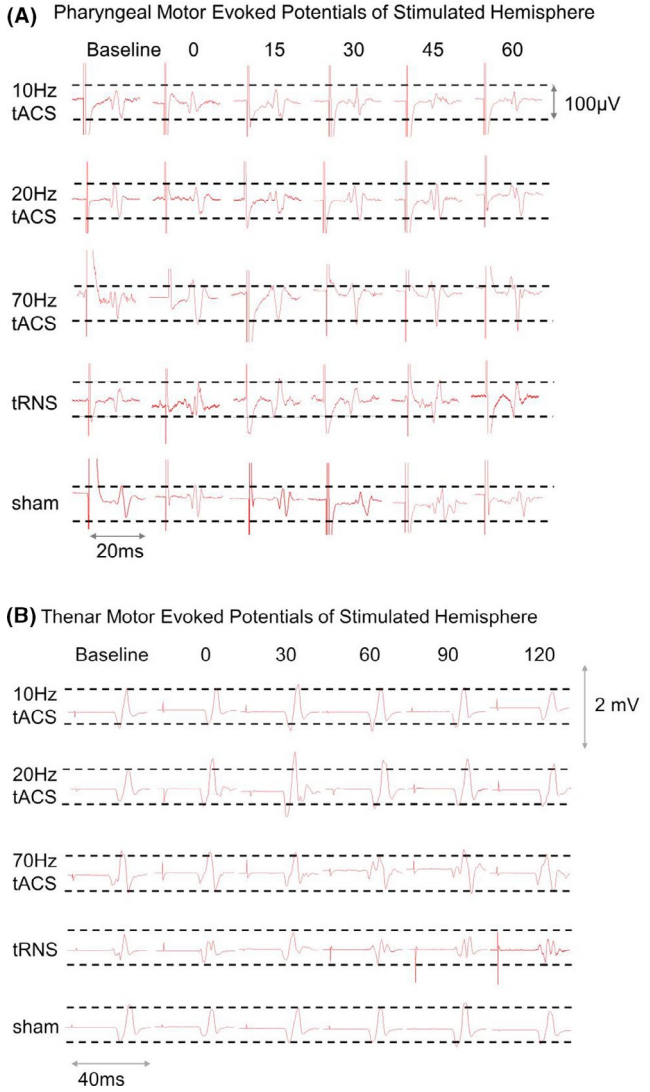

FIGURE 2 Representative PMEP and TMEP data traces from an individual participant for all stimulation parameters. (A) 70 Hz (gamma) tACS and tRNS increase PMEP amplitudes over 60 min in the stimulated hemisphere. (B) 20 Hz (beta) tACS increased ipsilateral TMEP amplitudes over 30–90 min. For display purposes, responses from the intermediate time points 15, 45, 75, and 105 min post-stimulation have been removed

regions of M1. For example, Laczó et al.[28] found that high-frequency tRNS applied over leg M1 increases human leg motor cortex excitability. Moveover, behaviorally, gamma tACS has been shown to improve both velocity and acceleration of visually triggered movements, compared with beta tACS and sham stimulation over M1.[18] Comparing theta, alpha, and beta frequencies, it was reported that gamma stimulation was the most effective frequency band for facilitating motor performance.[29,30] Hashimoto et al.[31] showed that the intrinsic gamma oscillation is focal and specific to swallowing function. Thus, a possible reason for the best effect on pharyngeal motor cortex excitability of gamma tACS could be related to the entrainment of ongoing gamma oscillations. By comparison, full-spectrum (0.1–640 Hz) and high-frequency (100–640 Hz) tRNS have similar effects on increasing cortical excitability.[5] While NIBS modes of action might differ, full-spectrum tRNS had an effect comparable to that of anodal tDCS and intermittent theta burst stimulation (iTBS), which are both normally excitatory, on MEP development over M1.[32] With regard to pharyngeal motor cortex stimulation, the efficacy of tDCS and rTMS for post-stroke dysphagia has shown promising results.[33,34] While the effects of pharyngeal electrical stimulation (PES) and 5 Hz rTMS were not evaluated in this experiment, we found that the degree of increased excitability of pharyngeal motor cortex by gamma tACS and full-spectrum tRNS is comparable to 5 Hz rTMS although appears slightly weaker than the size of effect reported for PES in previous studies.[8,9,23,35] Moreover, Doeltgen et al.[36] demonstrated that similar PMEP amplitude changes by anodal tDCS were able to improve swallowing function with increased bolus admittance across the upper esophageal sphincter. Our documented changes, therefore, have the potential to be translated into behavioral improvements and make the motor cortical application of gamma tACS and full-spectrum tRNS a promising adjunct to swallowing rehabilitation practice. Of interest to bilateral brain effects of NIBS, while the pharynx has bilateral cortical representation and transcallosal interactions between the two pharyngeal cortical areas are most likely synergistic, we found no change in excitability of the contralateral pharyngeal motor cortex. This is in accordance with a previous tDCS study, which also failed to demonstrate bilateral effects of tDCS to pharyngeal motor cortex.[23] We thus presumed that any effects on the non-stimulated hemisphere may be stimulus intensity-dependent with higher intensities of tACS and tRNS more likely to influence excitability transcallosally.

Alpha tACS at 1.5 mA has been noted to increase the cortical excitability of hand M1 in both young and elderly adults.[37] Wach et al.[4] reported that alpha tACS over M1 at 1 mA was significantly associated with shortening of the cortical silent period, causing reduced cortical inhibition. However, they did not find a significant effect on MEP amplitudes following 10 Hz tACS. This supports the idea that alpha tACS may interfere with inhibitory pathways or require greater stimulation. Therefore, we might propose that further increasing both intensity and duration of alpha stimulation would lead to greater (inhibitory) changes of excitability within the pharyngeal motor cortex.

Unlike the effects of tDCS which are driven by polarity-specific shifts of the resting membrane potential,[38] an advantage of tACS is that it permits physiological entrainment through frequency stimulation at nearly imperceptible current strengths. Frohlich and McCormick applied such AC fields to cortical slices of ferrets and found that current fields as low as 0.5 mV/mm were sufficient to modulate ongoing neural activity.[39] TACS was shown to manipulate the amplitudes of intrinsic oscillations as determined by EEG analysis,[16] and the changes of EEG oscillations were shown to have behavioral relevance.[40] Moreover, if the frequency of tACS is very close to the frequency of intrinsic brain oscillations, even very low currents can influence the oscillations amplitude, phase, and frequency.[1] Of relevance, changes in alpha, beta, and gamma frequency oscillations have been detected in the brain network of swallowing activities,

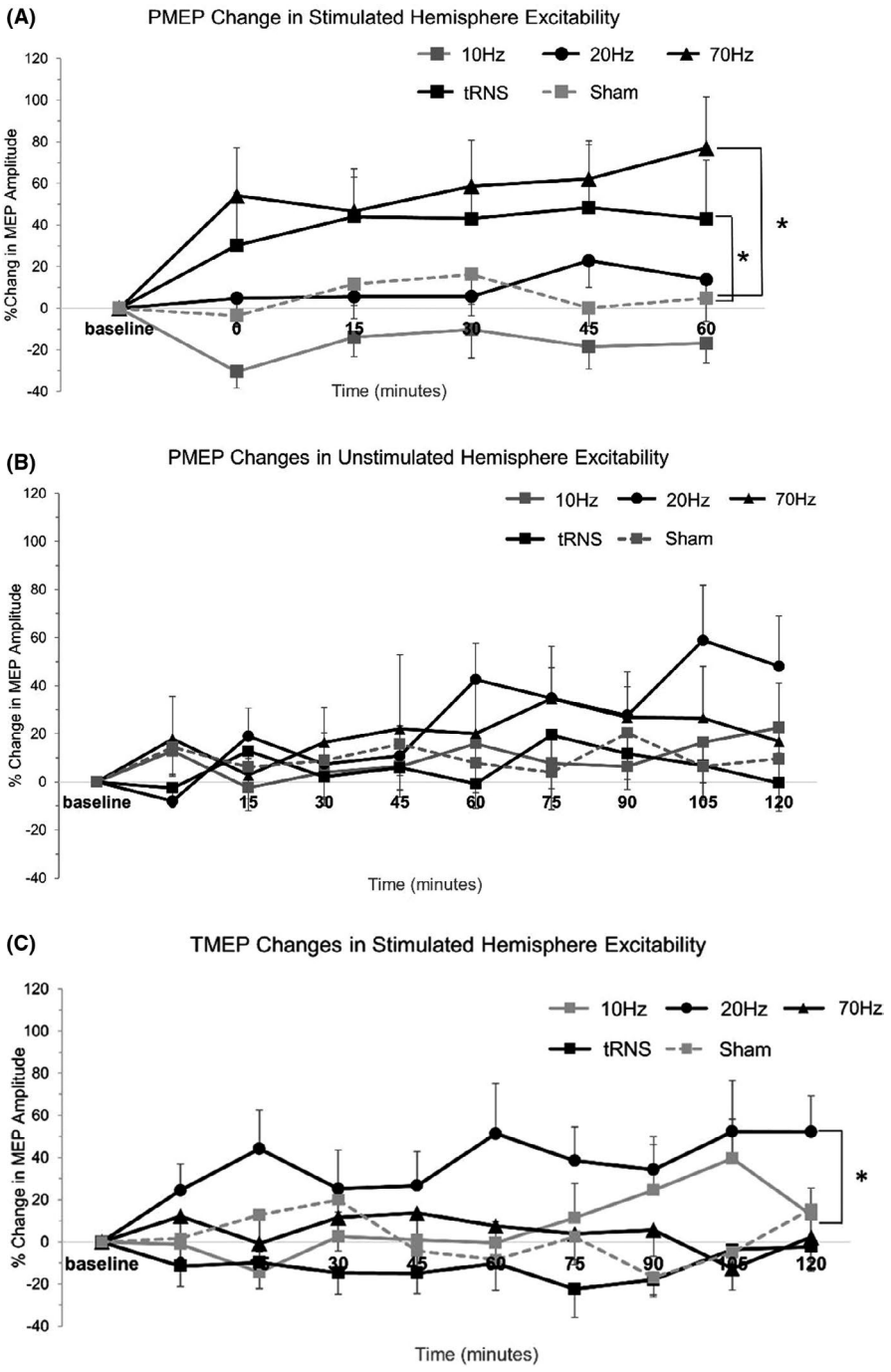

FIGURE 3 (A) Effects of different frequencies of tACS and tRNS on cortico-pharyngeal excitability in the stimulated hemisphere. 70 Hz tACS and tRNS increased pharyngeal cortical excitability compared to sham (*p = 0.005, *p = 0.027, respectively) which was sustained for 60 min; (B) there were no effects of interventions on cortico-pharyngeal excitability in the unstimulated hemisphere ($p > 0.05$); (C) effects of different frequencies tACS and tRNS on cortico-thenar excitability in the stimulated hemisphere. 20 Hz tACS induced excitatory effects on TMEP immediately which lasted for 2 h (*p = 0.006)

such as consuming thicker fluids and swallowing in the chin-tuck position.[41] The swallowing process requires an appropriate interaction between several CNS regions. It has been reported that alpha band oscillation is related more to sensory processes[17] and inhibition control,[42] whereas the beta rhythm is more closely tied to motor functions[43] and gamma oscillations play a role in a relatively late stage of motor control.[12] Therefore, our assumption is that the applied gamma band oscillations may be able to entrain the biological oscillations in swallowing networks and are potent enough to increase the excitability of neuronal populations in pharyngeal motor cortex. Although the current state of knowledge of the physiological mechanisms of how transcranial electric stimulation affects brain activity remains limited, a combined tACS-fMRI study demonstrated that gamma tACS induced motor performance enhancements which correlated with BOLD activity in the stimulated M1.[18] Furthermore, Guerra et al.[44] have reported indirect evidence that gamma tACS can reverse long-term depression (LTD)-like plasticity of the human primary motor cortex. It therefore seems plausible that a similar phenomenon occurs in different substrates of M1, including pharyngeal regions. From an intensity perspective, a recent meta-analysis reported that tACS enhances perceptual and cognitive performance in healthy volunteers, with >1 mA intensity displaying the highest probability of improving behaviors.[45] While we did not assess intensity specifically, the applied current intensity (1.5 mA) used in our

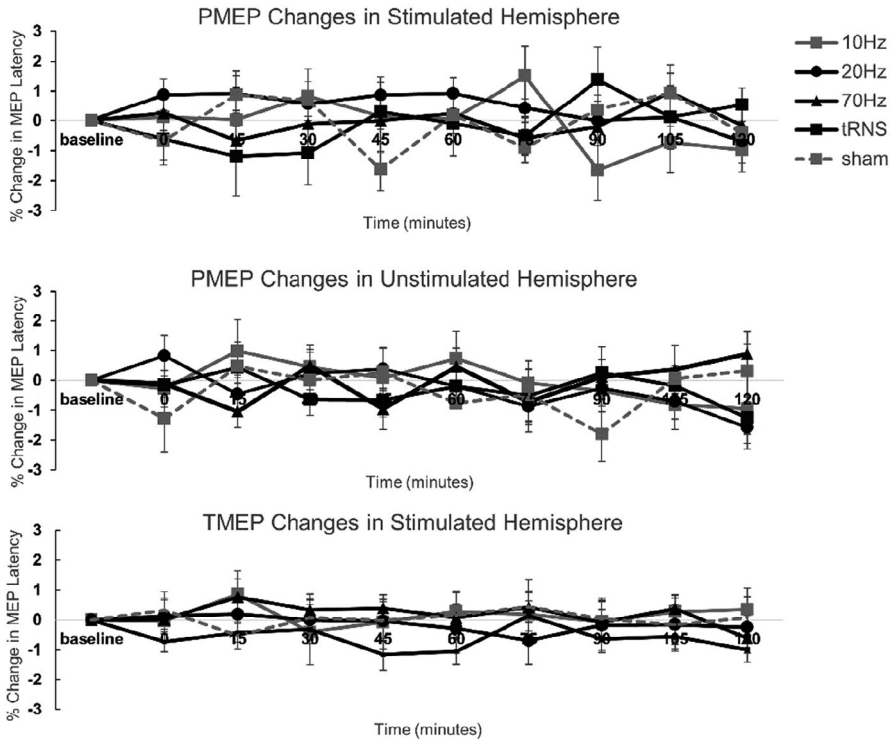

FIGURE 4 Percentage changes in PMEP and TMEP latencies. There were no effects of interventions on MEP latencies

study was able to facilitate the pharyngeal motor cortex, implying that higher intensities may be more preferential in this system.

Like tACS, the mechanisms underlying the effect of tRNS remain unclear with little or no animal studies to support insights into mechanism. However, tRNS over M1 has been shown to be comparable with the effects of anodal tDCS and tACS in altering human cortical excitability[32,46] and modifying performance.[22] Interestingly, the partial NMDA receptor antagonist D-cycloserine which blocks the effect of anodal tDCS had no significant effect on the excitability increases seen with tRNS.[47] Other studies, by contrast, have revealed that modulation of cortical excitability may be related to repeated opening of Na^+ channels[48] or stochastic resonance.[49] Our results have clearly found an effect of tRNS over pharyngeal M1, indicating these mechanisms may also happen in the swallowing network under certain conditions.

In transcranial stimulation studies, typically hand M1 is used as the main model for studying neuroplasticity or as target for treating neurological disorders, for example, after stroke.[9,23,50] This motor system was used as a control in our study, but intriguingly an increase of hand M1 excitability was found at beta tACS applied over pharyngeal hot spot, which differed from the frequencies that were effective in the pharyngeal motor cortex. In contrast, no changes of M1 hand area have been found in earlier tDCS studies in the swallowing system, using the same size electrodes (7 cm × 5 cm, 35 cm^2) and same hot spots (pharynx and thenar) to the present study.[9,23] A possible reason for the changes in thenar cortical excitability after tACS over the pharyngeal motor cortex could be the cortico-cortical links between the pharynx and hand motor areas. Previous studies suggest that the effects of transcranial electrical stimulation are not limited to the targeted brain region, and some therapeutic effects are probably mediated by distant brain areas. However, one feature argues against this possibility. If the effects on hand MEPs were due to cortico-cortical connectivity, we would have expected them to be modulated synchronously; by contrast, PMEPs and TMEPs were facilitated by different frequency settings and time durations. As such, given the electrode montage and the electrode size, we cannot exclude that current spread over to hand motor regions given their close proximity. Unlike tDCS, the electrical field reaching the cortex produced by tACS is typically less than 1 V/m which may be too weak to directly modulate the membrane potential and cause directly neural entrainment.[51,52] However, there is certainly evidence that beta tACS has differing properties to other frequencies. Indeed, a meta-analysis confirmed that beta tACS significantly increases M1 excitability[53] and these effects were completely abolished when an NMDAR antagonist was administered.[54] Therefore, future studies should explore the reasons for these site- and frequency-specific effects of tACS with a high-density montage.

4.2 | Sensory side effects of tACS and tRNS

In line with previous studies, our findings have shown that phosphenes and skin/scalp sensations are the two primary side effects and mainly occurred during alpha and beta tACS.[55,56] Current understanding about phosphenes indicates that they are generated in the retina by electricity spreading from the electrode locations near the eyes, since the retina is highly sensitive to current.[57,58] Although these side effects are generally less intense with tACS and tRNS than with tDCS,[59] it has the potential to affect the blinding procedure if the noticeable sensations are obviously different with active

stimulations. In our study, subjects seemed unable to distinguish between them, with sham having comparable sensory side effects to the active. Additionally, non-significant pre-post effects of sham electrical stimulation on corticospinal excitability have been identified.[26] As such, our data concur with previous studies, supporting the assertion that both tACS and tRNS appear to be safe and well-tolerated, with good blinding outcomes.

Our study does have some limitations. One deficiency of the study is that we only measured cortical excitability assessed with MEPs, whereas intracortical facilitation/inhibition, EEG monitoring, and fMRI may have helped further clarify the mechanism of oscillation entrainments. Secondly, our study did not look at behavioral measures of swallowing before and after stimulation to explore if the excitability changes translated into functional changes in swallowing performance. Additional research is needed to further investigate how tACS and tRNS affect swallowing behaviors and their effects as a treatment for patients with dysphagia.

In conclusion, gamma tACS and full-spectrum tRNS are able to enhance excitability within the areas of primary motor cortex controlling the pharynx in a frequency-dependent and site-specific manner. These techniques hold promise as potential treatments for neurological dysphagia.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Dr. M.Z. performed the studies in conjunction with Drs. I.C., A.S., and S.H.; Dr. M.Z. wrote the paper and analyzed the data along with Drs. I.C., A.S. Z.D., and S.H.; Drs. S.H. and Z.D. obtained funding. Dr. S.H. conceptualized and supervised the study, and helped with data interpretation and writing of the manuscript. All authors had access to the study data, and all approved the final manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ORCID

Mengqing Zhang https://orcid.org/0000-0002-1954-7802
Ivy Cheng https://orcid.org/0000-0001-5853-7976
Ayodele Sasegbon https://orcid.org/0000-0003-2050-0726
Shaheen Hamdy https://orcid.org/0000-0001-9640-7427

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SUPPORTING INFORMATION

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

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