

Short and long-term effects of pharyngeal  
electrical stimulation on swallowing  
performance in healthy humans

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## Abstract

Pharyngeal electrical stimulation (PES) has been found to facilitate voluntary swallowing. This study investigated how PES contributes to modulation of swallowing function in 12 healthy humans. In the involuntary swallowing test, distilled water was injected onto the pharynx at 0.1 mL/s and the onset latency of the first swallow was measured. In the voluntary swallowing test, subjects swallowed their own saliva as quickly as possible for 30 s and the number of swallows was counted. Voluntary and involuntary swallowing was evaluated before (control), immediately after and every 10 min after 10-min PES (75% of tolerated sensation, 5 Hz and 1 ms pulse) for 60 minutes. Voluntary swallowing test during PES was also conducted before (control) and 60 min after 10-min PES. The onset latency in the involuntary swallowing test tended to decrease and the number of voluntary swallows tended to decrease immediately after 10-min PES. There was a negative correlation between the changes. The number of voluntary swallows with PES significantly increased 60 min after 10-min PES as compared with baseline. Repeat 10-min PES over 5 days significantly facilitated voluntary swallowing, with significant increases in the number of voluntary swallows. Involuntary swallowing function was not affected by PES. The results suggest that PES may have a long-term facilitatory effect on initiation of voluntary swallowing even in healthy humans but not on peripherally evoked swallowing. Daily repeat application of PES may also be useful in increasing the number of voluntary swallows. Physiological implications for the modulation and possibility of using the methods in dysphagic patients are discussed.

## Key words

pharyngeal electrical stimulation; voluntary swallow; involuntary swallow; dysphagia

## INTRODUCTION

Swallowing involves complex sensorimotor neural components. The complexity of swallowing may be explained by the fact that swallowing has a number of functions, including propelling the food bolus from the oral cavity into the stomach through the pharynx and the esophagus, and also protecting the upper respiratory tract by cleaning the larynx and pharynx, and hence preventing choking or aspiration of secretions or food (6, 17, 28, 31). In addition, to complete normal swallowing movements, more than 25 pairs of related muscles in the orofacial, pharyngeal, laryngeal, and esophageal regions must be activated bilaterally and in coordination (6, 17, 28). Underlying motor patterns of swallowing are programmed by the so-called central pattern generator (CPG) in the medulla oblongata, and both the peripheral and central inputs into the CPG can trigger swallowing (17, 28); swallowing can be initiated either involuntarily or voluntarily.

Peripherally evoked swallowing can be initiated by mechanical or chemical stimulation in the oropharynx or larynx. Sensory regions that elicit pharyngeal swallowing include the soft palate, uvula, dorsal tongue surface, faucial pillars, dorsal pharyngeal wall, pharyngeal surface of the epiglottis, and the glossoepiglottic sinus (34, 40, 41, 43, 44). The superior laryngeal nerve (SLN) and pharyngeal branch of the glossopharyngeal (GPNph) nerve innervating those areas are known to be the most responsive nerves to electrical stimulation, and trigger repetitive pharyngeal swallowing, i.e., the swallowing reflex, even in anesthetized animals (22, 48, 49). Because initiation of the swallowing reflex is not interrupted after ablation of the cortex, peripheral inputs may be effective enough to initiate swallowing within the neural circuit of the brainstem (48). One can therefore expect that

electrical stimulation of the nerves or regions is also able to activate and/or facilitate the activation of the swallowing CPG in humans.

A previous study showed that repetitive pharyngeal electrical stimulation (PES) facilitated voluntary swallowing in conscious humans (51). The result was expected because the stimulated areas were innervated by the SLN or GPNph. However, initiation of swallowing by this method seemed to be difficult compared with that in anesthetized animals (46, 51). Takatsuji et al. (46) reported that continuous PES failed to elicit repetitive swallowing although the first swallow was successfully evoked following stimulation. Possible reasons for the discrepancy between animal and human studies might be: (1) a species difference; (2) a difference in the stimulus conditions, in that surface stimulation was not effective in fully activating the neural network in humans; or (3) a difference in animal/human physiology, in that cortical activity inhibited the neural circuit of swallowing in either the cortical/subcortical areas or the brainstem in conscious humans.

One should consider that even subliminal stimulation may change cortical activity. Kern et al. (20) studied the cerebral cortical functional magnetic resonance imaging (fMRI) response to subliminal, liminal, and supraliminal rectal distention and showed distention level-dependent signal changes. Previous studies demonstrated that PES produced long-lasting changes in swallowing-associated motor cortical excitability. Fraser et al. and Power et al. showed that, after 10-min pharyngeal and oral electrical stimulation, corticobulbar excitability evaluated by cortical transcranial magnetic stimulation (TMS) evoked potentials in the pharyngeal/esophageal muscles were increased 60 min after stimulation (7, 8, 35). Interestingly, the ideal stimulation frequency for facilitation was different between pharyngeal and oral stimulation. A facilitatory effect was noted after stimulation at 5 Hz, at 75% of maximum tolerated sensation for the former, and at 0.2 Hz, at

75% of maximum tolerated sensation for the latter. Regarding PES, the long-term effect was also observed in brain imaging data. Fraser et al. (7) reported that the cortical blood oxygenation level-dependent fMRI signal showed greater bilateral functional activation within the sensorimotor cortex (BA 3/4) 60 min after 10-min PES compared with no PES.

A few studies have investigated the effect of peripheral electrical stimulation on swallowing behavior. After 10-min oral electrical stimulation at 0.2 Hz, all swallowing measures, including oral transit time, swallowing response time, defined as the time interval between the presentation of the bolus at the hypopharynx and laryngeal elevation, airway closure duration, and cricopharyngeal opening time remained unaffected, although corticobulbar excitability significantly increased 60 min after 10-min stimulation (35). On the other hand, after 10-min PES, pharyngeal transit time, swallowing response time, and aspiration were significantly improved in acute stroke patients (7, 16). Thus, although PES may eventually facilitate corticobulbar excitability, it has not been fully clarified how pharyngeal stimulation contributes to changes in swallowing behavior, particularly initiation of swallowing movements.

To this end, we investigated the short- and long-term effect of PES on swallowing function by measuring the onset latency of the first swallow during water infusion at a very slow rate (0.1 mL/s) to represent the swallowing reflex function, and the number of voluntary swallows for 30 s to represent corticobulbar excitability for initiation of voluntary swallowing. The aim of our study was to evaluate the effects of PES on the short- and long-term changes in swallowing behaviors in terms of initiation of involuntary and voluntary swallowing in healthy human subjects.

## MATERIALS AND METHODS

*Participants.* Thirteen healthy male adults (mean age  $\pm$  SD: 27.0  $\pm$  6.1 years; age range: 22–37 years) participated in the study. Informed consent was obtained from all participants, and no subject had a history of pulmonary disease, neurological disease, musculoskeletal disorders, speech disorders, voice problems, or masticating or swallowing problems. The experiments were approved by the Ethics Committee of the Faculty of Dentistry, Niigata University (25-R33-11-25).

*Electrophysiological recordings.* To monitor swallowing events, electromyographic (EMG) and electroglottographic (EGG) activities were recorded according to previous studies (30, 33, 36, 47, 50). Bipolar surface EMG electrodes (WEB-1000; Nihon Kohden, Tokyo, Japan) were attached to the skin over the anterior surface of the digastric muscle on the left side, and EMG signals were detected in the suprahyoid muscle group. They were filtered and amplified (low, 30 Hz and high, 2 KHz) (WEB-1000; Nihon Kohden, Tokyo, Japan) to remove movement-related artifacts. Bipolar surface EGG electrodes were positioned on either side of the thyroid cartilage and the signals were amplified (EGG-D200; Laryngograph, London, UK). Amplified EMG and EGG signals were stored through an interface board (PowerLab; ADInstruments, Colorado Springs, CO, USA) on a personal computer. The sampling rate was 10 kHz. Data analysis was performed using the PowerLab software package (LabChart6; ADInstruments, Colorado Springs, CO, USA). As with those signals, button-pressing was also recorded. In this procedure, the subject was instructed to hold a button in his hand and press it immediately after each swallow.

*Pharyngeal electrical stimulation.* For PES, catheter electrodes (TK210-107b; Unique Medical Co., Ltd., Tokyo, Japan) were developed. The catheter housed two ring electrodes made of platinum for electrical stimulation. The most distal electrode was 3 mm from the tip

of the catheter, with a distance of 13 mm between the electrodes. The stimulation site was at the lateral wall of the laryngopharynx, at the level of the pyriform sinus, and was confirmed by videoendoscopy. Bipolar surface electrical stimulation (1 ms pulse duration; 5 Hz) was delivered through cables connected to an electrical stimulator (SEN3401, Nihon Kohden, Tokyo, Japan). To determine the intensity of the stimulus, the current was increased by 0.1 mA every 5 s. Once thresholds for perception (STper) and tolerability (STtol) were determined by the subject's cue, the 75% maximum tolerated intensity was calculated as  $ST_{per} + 0.75 (ST_{tol} - ST_{per})$ .

*Assessment of swallowing performance.* Two tests were conducted: an involuntary swallowing test and a voluntary swallowing test. In the involuntary swallowing test, a fine Teflon tube of 2.67 mm outer diameter (NSC-8(TA2)C; Nipro, Osaka, Japan) was inserted transnasally into the pharynx. The tip of the tube was confirmed to be positioned on the posterior wall of the oropharynx at the level of the epiglottic vallecula by videoendoscopic observation. Distilled water (20–25°C) was delivered through the tube using an infusion pump (KDS100; Muromachi Kikai Co., Ltd., Tokyo, Japan) at 0.1 mL/s. Subjects were instructed to swallow whenever ready to swallow and not to make any effort in voluntary swallowing. The onset latency of the first swallow was measured (Fig. 1).

In the voluntary swallowing test (Fig. 2), subjects were instructed to perform repetitive swallowing as quickly as possible over 30 s and the number of swallows was counted (51). Because a previous study demonstrated that the number of voluntary swallows in a certain time differed between young and old subjects (32), we did not recruit any elderly subjects in the present study. Prior to each test, the subject was asked to swallow his own saliva 10 s before recording to clear the saliva in the oral and/or pharyngeal cavity.

*Experimental protocol 1: One-day stimulation.* To reduce a possible effect of circadian variations or the environment on swallowing performance, experiments were performed at the same time of day in each individual in an air-conditioned room, where room temperature was kept at 20–24°C and humidity was kept at 40–70%. Subjects were asked to refrain from eating, drinking, smoking, and brushing their teeth for at least 60 min before the experiment. The subjects were seated comfortably and remained upright throughout the study. A catheter to deliver electrical stimulation and a tube to infuse solution into the pharynx were inserted transnasally. A minimum of 5 min was allowed for the subjects to become accustomed to the catheter. The stimulus threshold was determined as mentioned above.

The experimental protocol is shown in Fig. 3. At an interval of 2 min, involuntary and voluntary swallowing tests were performed. In the involuntary swallowing test, the onset latency of the first swallow evoked was measured. Two voluntary swallowing tests were conducted without and with PES (Fig. 2) at an interval of 2 min. The number of swallows was measured in the two tests. The onset latency in the involuntary swallowing test and the number of voluntary swallows in 30 s without and with PES were regarded as control values.

After these baseline measurements to record control values, PES was carried out at intensities of 75% maximum tolerated intensity for 10 min at a frequency of 5 Hz and pulse duration of 1 ms (stimulation group, n=9) or there was no stimulation (sham group, n=4). In the sham group (Fig. 3), all the procedures were the same as for the stimulation group but 10-min PES was not delivered. During the 10-min PES, subjects were instructed to keep quiet but there was no limit on spontaneous saliva swallowing. Following 10-min PES or no stimulation, involuntary and voluntary swallowing tests were performed immediately after PES and at 10-min intervals for 60 min. Finally, a voluntary swallowing test with PES was performed.

*Experimental protocol 2: Five-day PES application.* Of nine subjects in the stimulation group, six received 5 days of 10-min PES. The experimental procedure each day was the same as that of the 1-day application. Although it was predictable that thresholds for perception (STper) and tolerability (STtol) were variable between days, the stimulus current was not changed throughout the 5-day experiment in each individual.

*Data analysis.* In the analysis of the number of swallows in the voluntary swallowing test without and with PES in protocol 1, two control values were first compared using the paired *t*-test to evaluate the immediate effect of PES on the number of voluntary swallows. Then the individual mean values of the onset latency of the first swallow in the involuntary swallowing test and the number of swallows in the voluntary swallowing test without PES were compared between different times by using one-way repeated-measures analysis of variance (ANOVA). The number of swallows with PES was compared between control and 60 min after 10-min PES using the paired *t*-test. In the present study, we did not compare the values between stimulation and sham groups because the sample size in the sham group was low.

In the analysis of the data of protocol 2, as for the data of protocol 1, the mean values of the onset latency of the first swallow and number of swallows without PES were compared between different days by one-way repeated-measures ANOVA. The number of swallows with PES was compared between control and 60 min after 10-min PES by the paired *t*-test. Statistical significance was set at  $p < 0.05$ . All values are expressed as mean  $\pm$  SD.

## RESULTS

*Stimulus threshold of perception and tolerability and immediate effects.* The threshold of

the stimulus current for perceived and tolerated sensations varied among subjects at  $0.9 \pm 1.3$  mA (n=13) and  $3.1 \pm 2.6$  mA (n=13), respectively. There was no significant difference between the stimulation and sham groups in the perceived and tolerated sensations:  $1.1 \pm 1.6$  mA (stimulation group, n=9) vs  $0.6 \pm 0.4$  mA (sham group, n=4) for perceived sensation and  $3.3 \pm 2.9$  mA (stimulation group, n=9) and  $2.4 \pm 1.7$  mA (sham group, n=4) for tolerable sensation.

The number of swallows in the voluntary swallowing test in controls without and with PES was  $7.8 \pm 2.0$  (n=13) and  $9.8 \pm 3.1$  (n=13), respectively (Fig. 4), a significant difference ( $p < 0.05$ ). In the stimulation group, the number of swallows was  $7.5 \pm 2.4$  (n=9) without PES and  $9.3 \pm 2.6$  (n=9) with PES, a significant difference (Fig. 4). Partly because the number of subjects was low in the sham group, no significant difference in the number of swallows was found between without PES ( $7.8 \pm 1.3$ , n=4) and with PES ( $10.8 \pm 4.3$ , n=4) (Fig. 4). These results showed that repetitive PES may facilitate voluntary swallowing in terms of an increase in the number of swallows in a certain time as reported before (51). Because there were wide variations in the threshold of perceived and tolerated sensation among the subjects, the relationship between the stimulus current and increase in the number of swallows was evaluated. No significant correlations were found, although there was a trend for a positive correlation (Fig. 5).

*Effects of PES in the stimulation group.* All the data are summarized in Fig. 6.

Immediately after 10-min PES, the onset latency of the first swallow tended to be longer compared with control, but there was no significant difference. The onset latency gradually decreased and returned to baseline values at 60 min. The number of voluntary swallows tended to decrease immediately after 10-min PES compared with control, although there was no significant difference, and this was followed by an increase at 60 min. Interestingly,

there was a negative correlation between the increase in the onset latency of the first swallow and the decrease in the number of voluntary swallows immediately after 10-min PES (Fig. 7A). Regarding the effect of stimulus intensity or the number of evoked swallows during 10-min PES on the onset latency of the first swallow and number of voluntary swallows immediately after 10-min PES, only the former was affected by the stimulus intensity; the larger the stimulus intensity, the greater the increase in onset latency of the first swallow (Fig. 7B).

The number of swallows with PES before and 60 min after 10-min PES was  $9.3 \pm 2.6$  (n=9) and  $10.8 \pm 2.0$  (n=9), respectively (Fig. 6C). Thus, 60 min after 10-min PES, there was a small but significant increase in the number of swallows with PES.

As mentioned above, the stimulus intensity varied among the subjects. In addition, the number of evoked swallows during 10-min PES also varied widely, and the mean number of swallows was  $33.4 \pm 14.1$  (n=9), ranging from 12 to 55. Because it could be predicted that the stimulus intensity and/or number of evoked swallows during 10-min PES affected the following facilitation of initiation of voluntary swallows, we evaluated the relationship between stimulus intensity, evoked swallows, and facilitation of voluntary swallowing 60 min after 10-min PES. There was no significant correlation (Fig. 8), and the results suggest that facilitation of voluntary swallowing with PES was not directly related to the stimulus intensity applied to the pharynx or to the number of evoked swallows during 10-min PES.

*Sham group.* Fig. 9 summarizes the results in the sham group. With no 10-min PES, there was no significant difference in the onset latency of the first swallow over time. Although there was no significant difference among the values, the number of swallows both without and with PEG gradually decreased over time (Fig. 9B and C).

*Effects of 5-day application of PES on swallowing performance.* Of nine subjects in the

stimulation group, six received repeat 10-min PES daily for 5 days (Fig. 10). The onset latency of the first swallow did not exhibit any significant changes. The number of swallows in the voluntary swallowing test gradually increased over 5 days. The number of swallows without PES was  $8.2 \pm 2.7$  on the first day,  $8.5 \pm 2.3$  on the second day,  $9.0 \pm 2.7$  on the third day,  $8.3 \pm 2.7$  on the fourth day, and  $9.3 \pm 2.9$  (n=6) on the fifth day, with no significant difference among the days. The number of swallows 60 min after 10-min PES was  $9.1 \pm 3.4$  on the first day,  $8.3 \pm 3.0$  on the second day,  $8.7 \pm 2.9$  on the third day,  $8.8 \pm 3.4$  on the fourth day, and  $10.7 \pm 4.3$  (n=6) on the fifth day. Although differences were small, they were significant between the second and fifth day and between the third and fifth day.

As well as the number of voluntary swallows without PES, that with PES also increased over 5 days. The number of swallows in controls was  $9.3 \pm 3.3$  on the first day,  $10.5 \pm 2.9$  on the second day,  $10.3 \pm 3.5$  on the third day,  $11.9 \pm 2.9$  on the fourth day, and  $12.8 \pm 4.0$  (n=6) on the fifth day, with a significant difference between the first and fifth day. The number of swallows 60 min after 10-min PES was  $11.1 \pm 2.1$  on the first day,  $11.0 \pm 2.3$  on the second day,  $11.9 \pm 2.9$  on the third day,  $12.3 \pm 3.7$  on the fourth day, and  $13.7 \pm 4.8$  (n=6) on the fifth day, with a significant difference between the second and fifth day.

## DISCUSSION

In the present study, the effects of 10-min PES on swallowing performance were investigated in healthy humans. The onset latency of involuntary swallowing representing the swallow reflex function tended to increase and the number of voluntary swallows tended to decrease immediately after 10-min PES. Although these changes were small and not significant, a negative correlation between the changes in involuntary and voluntary

functions was noted. On the other hand, 60 min after 10-min PES, the number of voluntary swallows gradually increased. In particular, the number of swallows with PES significantly increased compared with control, while the onset latency of involuntary swallowing only returned to baseline values after 60 min. Daily application of PES also resulted in an increase in the number of voluntary swallows while the onset latency of involuntary swallows was not affected.

*Mechanism of PES.* To activate the swallowing CPG and/or to evoke swallowing movements, both supramedullary and peripheral inputs exist (6, 17). Regarding peripheral inputs, mechanical stimulation or chemical stimulation applied to the pharynx or larynx is known to evoke the swallowing reflex in animals (19, 39, 43, 44) and humans (24, 34, 38, 43, 44). In particular, sour tastes delivered to those regions are effective (15, 38), as is water (21, 30, 38, 39, 53). In addition to natural stimulation, electrical stimulation of the SLN innervating pharyngeal and laryngeal regions can easily evoke the swallowing reflex, and was routinely used to induce either sole or rhythmic swallowing with less fatigue in dogs (5), rabbits (23), and cats (27). In our study, repetitive electrical stimulation was delivered into the laryngopharyngeal mucosa, which is mainly innervated by the SLN, so that the swallowing CPG might be directly activated. Although the swallowing frequency was not precisely analyzed in the present study, repetitive swallowing was not observed during 10-min PES unlike in animal studies. In animals, higher stimulus frequencies between 30 and 50 Hz resulted in quick initiation of the first swallow and repeat swallows (5, 22). Our previous studies also employed 30 Hz stimulation to increase swallowing frequency in the voluntary swallowing test (51). On the other hand, 5 Hz stimulation was sufficient to increase the number of voluntary swallows with PES, probably as a result of simultaneous inputs from both supramedullary and peripheral afferent fibers. One of the aims of the present study was

to evaluate the long-term effect of PES on swallowing performance, including involuntary and voluntary swallowing, as well as immediate and short-term effects. Changes in sensory inputs can produce persistent changes in the organization of sensory and motor areas of the cerebral cortex (18, 52). Previous human and animal studies reported that a reduction in sensory feedback can induce changes in motor representation in the cerebral cortex (1-3, 37). Fraser et al. (7) demonstrated that changes in somatosensory input can remodel human cortical motor organization in humans. The authors showed that cortical TMS-evoked motor potentials in the pharyngeal and esophageal muscles were highly dependent upon the frequency, intensity, and duration of PES, with 5 Hz, 0.2 ms pulse duration and 75% maximal tolerated intensity for 10 min inducing stronger cortical activation. Numerous studies followed this method and showed changes or improvement in swallowing behaviors (7, 8, 13, 16, 26, 45). Although the current study generally followed the same method, the pulse duration in our study was 1 ms, which was much longer. As a result, the mean stimulus current of 75% of maximum tolerated sensation was much lower than in previous studies. We will have to examine in future how the difference in pulse duration affects facilitation.

*Short-term effect of PES.* Immediately after 10-min PES, both involuntary and voluntary swallowing functions tended to be inhibited and there was a significant correlation between the reduction in involuntary and voluntary swallowing functions.

In our previous study in anesthetized rats, repetitive swallowing gradually decreased during continuous SLN stimulation (48). The reduction was not related to sensory feedback but to the magnitude of the sensory input; the reduction was dependent on the stimulus frequency, and prior subthreshold SLN stimulation also reduced time to initiation of swallowing. In addition, the reduction was not affected by decerebration, suggesting that

adaptation to PES occurred in sensory afferent nerve firing and/or trans-synaptic responses as part of the swallowing CPG following continuous SLN stimulation. The results were identical to those in the present study. Inhibitory trend of both involuntary and voluntary swallowing functions was not affected by the number of swallows evoked during 10-min PES but that of the involuntary swallow was affected by the stimulus intensity. In the sham group, involuntary and voluntary swallowing function was not changed immediately after 10-min of no stimulation. Taken together, it can be assumed that the common component in the neural network responsible for both involuntary and voluntary swallowing functions was affected by 10-min PES.

Previous studies reported reduced H reflex responses in the soleus muscle in response to electrical stimulation of the subthalamic nucleus or magnetic stimulation (4, 9). The reduction was attributed to activation of an inhibitory descending pathway during prolonged stimulation. It may also be possible that continuous stimulation activated an inhibitory neural network in the basal ganglia leading to a temporary refractory period following continuous PES, although we do not have any direct evidence for this.

*Long-term effect of PES.* To my knowledge, the present study is the first to clarify the long-term effect of PES on swallowing performance in terms of facilitation of initiation of voluntary swallowing. In addition, a daily stimulation facilitatory effect was also observed. In contrast to the effect on voluntary swallowing, the involuntary swallowing function was not affected.

Numerous studies reported long-term 1-day and repeat daily effects of PES in healthy subjects and dysphagic patients. In healthy subjects receiving PES, TMS-evoked potentials in the pharyngeal and esophageal muscles were increased over time (7, 8, 12, 13, 16). In addition, at the same time, the number of brain sites evoking a response in the pharyngeal

and esophageal muscles by TMS increased bilaterally in the cortex, and an increase in the areas of voxel activation was observed in the lateral sensorimotor cortex by fMRI (7).

Sunstrup et al. performed whole-head magnetoencephalography and observed a prominent reduction in event-related desynchronization during voluntary swallowing in sensorimotor brain areas 45 min after PES (45). As with the brain imaging data, the authors showed that volume per swallow and swallowing capacity significantly increased following PES. In dysphagic patients, electrophysiological data showed a marked increase in pharyngeal corticobulbar excitability and topographic representation in the undamaged hemisphere, and there was also an improvement in swallowing function, including shorter pharyngeal transit time, shorter onset latency of pharyngeal swallowing during voluntary swallowing, and decreased frequency of aspiration (7). On the other hand, Jayasekeran et al. provided positive data from daily use of PES in post-stroke patients (16). The authors employed 10-min PES and showed that 3-day use of PES produced improved airway protection, reduced aspiration, and improved feeding status. These results were identical to those of the current study in that the number of voluntary swallows increased 60 min after 10-min PES, and that daily repeat application led to an increase in voluntary swallows only.

In the present study, although the number of swallows during 10-min PES and the stimulus current varied widely among subjects, they were not related to the facilitatory effect of voluntary swallowing. For the former, PES-evoked swallowing during 10-min stimulation may involve only the reflex network within the brainstem but not in the cortex or other long loop reflex networks. For the latter, although no subject reported any uncomfortable feelings, the catheter touched the mucosa in the nasal and pharyngeal cavities throughout the recordings, which might cause a subject-dependent effect on pain sensation or discomfort

(51). It remains unclear whether the stimulus intensity for perception and tolerability in each individual represented the actual peripheral inputs.

In humans, numerous studies employed TMS (10, 11) and brain imaging (14, 25, 29) to identify swallowing-associated cortical areas, and found that there may be multiple cortical foci including the lateral pericentral and premotor cortices, frontal operculum and insula. Although the current experiments did not address the mechanism of the sensory driven effects and why they were able to facilitate voluntary swallowing even in healthy subjects, it can be suggested that the effects on those swallowing-related cortical excitabilities may be related to such phenomena as long-term potentiation. In fact, the time course of the facilitatory effect was similar to that seen in another study on the human motor cortex (42). Nevertheless, the precise mechanisms for the modulation of the swallowing function obtained in the present study cannot be addressed in experiments on the human cortex, and should be addressed in further studies.

*Clinical implications.* In the present study, the number of voluntary swallows varied greatly from subject to subject, but facilitation 60 min after 10-min PES and after daily repeat PES was obvious, suggesting that sensory activation compensates for the difficulty in initiating swallowing via the central neural mechanism. From the current results, clinicians may also be interested in the difference in such swallowing ability not only in healthy individuals, but also in patients with dysphagia caused by aging or disease. Most patients with dysphagia cannot perform repetitive swallowing like healthy humans. Although where and how the neural network is involved in impairment or recovery of swallowing performance in those patients remains unclear, 10-min PES may be a simple and safe method to treat such patients and deserves further investigation.

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## Figure legends

Fig. 1. Measurement of onset latency of the first swallow in the involuntary swallowing test. Suprahyoid electromyogram (EMG) was recorded with infusion of distilled water at 0.1 mL/s into the pharynx. Time duration between the start of the infusion and the peak of the EMG burst during involuntary swallowing was measured. A swallowing event was also identified by electroglottographic (EGG) signals. fEMG, filtered (rectified and smoothed) EMG.

Fig. 2. Example of EGG and suprahyoid EMG recordings in the voluntary swallowing test without and with pharyngeal electrical stimulation (PES). Swallowing events were identified by EGG and EMG bursts as well as the subject pressing a button (closed triangle). In this case, the number of swallows for 30 s was increased with PES from 10 to 12.

Fig. 3. Experimental protocol of the study. Involuntary and voluntary swallowing tests (Tests) were conducted followed by a voluntary swallowing test with PES (Test w/PES) at an interval of 2 min. Then 10-min PES or no stimulation was delivered. Immediately afterwards and after every 10 min up to 60 min, both involuntary and voluntary swallowing tests were performed in the stimulation and sham groups. Then, a voluntary swallowing test with PES was performed.

Fig. 4. Immediate effect of PES. The mean number of swallows in the voluntary swallowing test without and with PES was  $7.8 \pm 2.0$  (n=13) and  $9.8 \pm 3.1$  (n=13), respectively. They were  $7.8 \pm 1.3$  (n=4) without PES and  $10.8 \pm 4.3$  (n=4) with PES in the sham group and  $7.5 \pm 2.4$  (n=9) without PES and  $9.3 \pm 2.6$  (n=9) with PES in the stimulation group. Overall and

in the stimulation group, there was a significant difference between without and with PES.

\* $p < 0.05$ .

Fig. 5. Relationship between stimulus intensity and facilitatory effect on the number of swallows in the voluntary swallowing test. There was a trend for a positive correlation.

$y = 0.4x + 0.9$ ,  $R^2 = 0.16$ ,  $p = 0.17$ .

Fig. 6. Long-term effect of 10-min PES in the stimulation group. (A) Onset latency of the first swallow was slightly longer immediately after 10-min PES and returned to the baseline level after 60 min. (B) The number of voluntary swallows slightly decreased immediately after 10-min PES and gradually increased over 60 min. (C) The number of voluntary swallows with PES significantly increased compared with baseline. \* $p < 0.05$ .

Fig. 7. Relationship between difference in the onset latency of the first swallow, in the number of voluntary swallows, and the stimulus intensity. The relationship between the difference in the onset latency of the first swallow and that of the number of voluntary swallows is shown in A. X values represent the increase in onset latency of the first swallow between before and immediately after 10-min PES. Y values represent the increase in the number of voluntary swallows between before and immediately after 10-min PES. There was a significant negative correlation.  $y = -0.3x + 0.2$ ,  $R^2 = 0.7436$ ,  $p < 0.05$ . The relationship between the difference in the onset latency of the first swallow and stimulus intensity is shown in B. X values represent the stimulus intensity in the 10-min PES. Y values represent the increase in the onset latency of the first swallow between before and immediately after 10-min PES. There was a small but significant positive correlation.  $y = 0.9x - 1.1$ ,  $R^2 = 0.4756$ ,

$p < 0.05$ .

Fig. 8. Relationship between stimulus intensity, evoked swallows during 10-min PES, and increase in the number of swallows in the voluntary swallowing test with PES. There was no significant correlation between stimulus intensity and number of swallows (*A*), between stimulus intensity and increase in number of swallows (*B*) and between the number of swallows during 10-min PES and the increase in the number of swallows in the voluntary swallowing test (*C*).

Fig. 9. Long-term effect in the sham group. (*A*) Onset latency of the first swallow did not exhibit any significant changes. (*B, C*) The number of voluntary swallows without and with PES tended to decrease at 60 min, although there was no significant difference.

Fig. 10. Long-term effect of 5-day repetition of PES in the stimulation group. (*A*) The onset latency of the first swallow was not significantly changed after 5 days. (*B*) The number of voluntary swallows increased after 5 days. There was a significant difference between the second and fifth day and between the third and fifth day at 60 min after 10-min PES (closed circles). (*C*) The number of voluntary swallows with PES increased after 5 days. There was a significant difference between the first and fifth day in controls (open circles) and between the second and fifth day at 60 min after 10-min PES (closed circles). \* $p < 0.05$

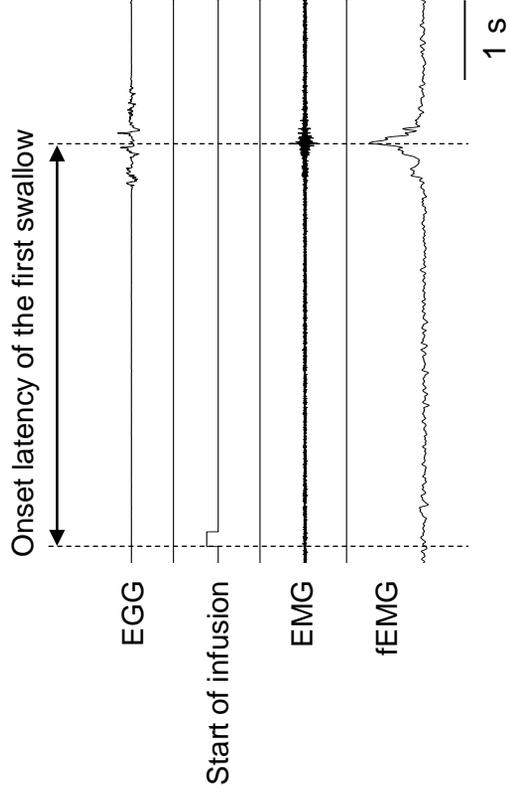


Fig. 1

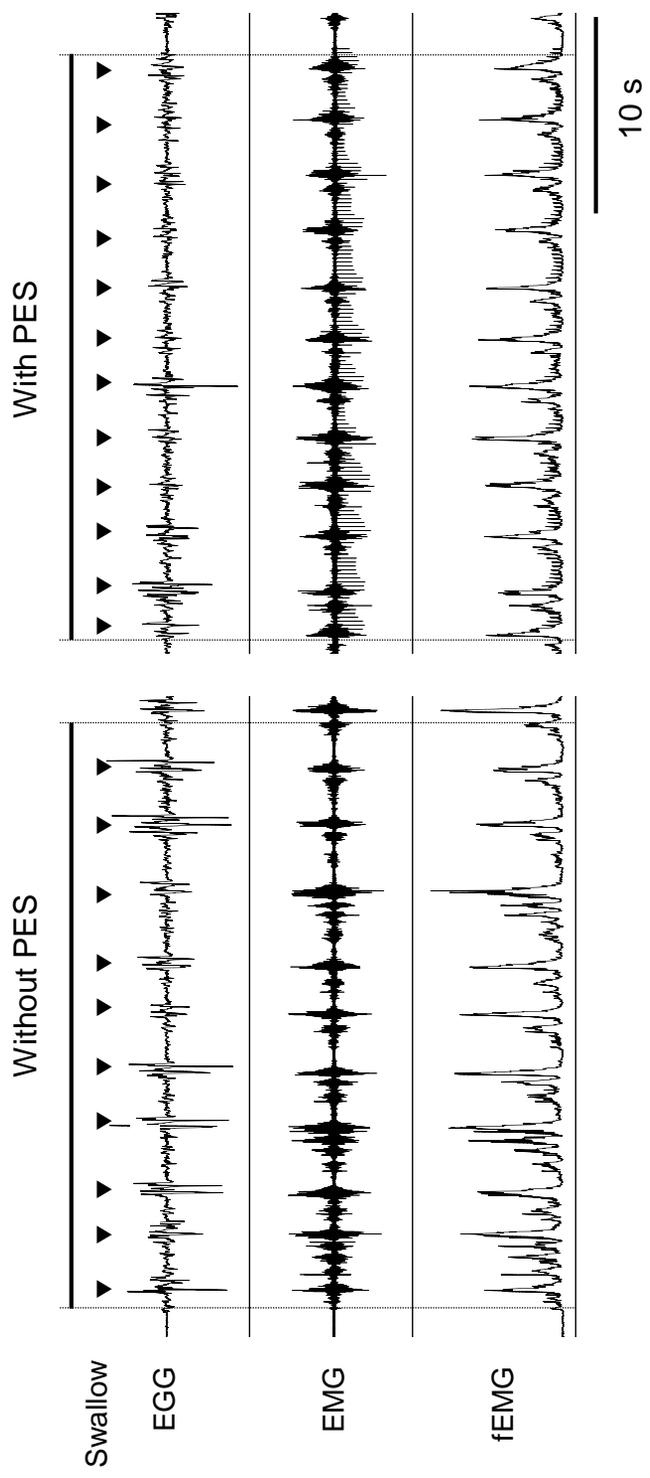


Fig. 2

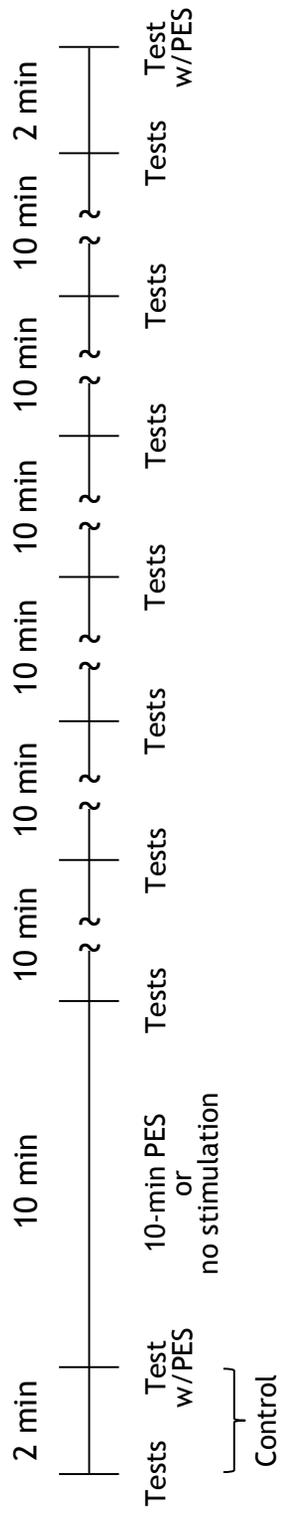


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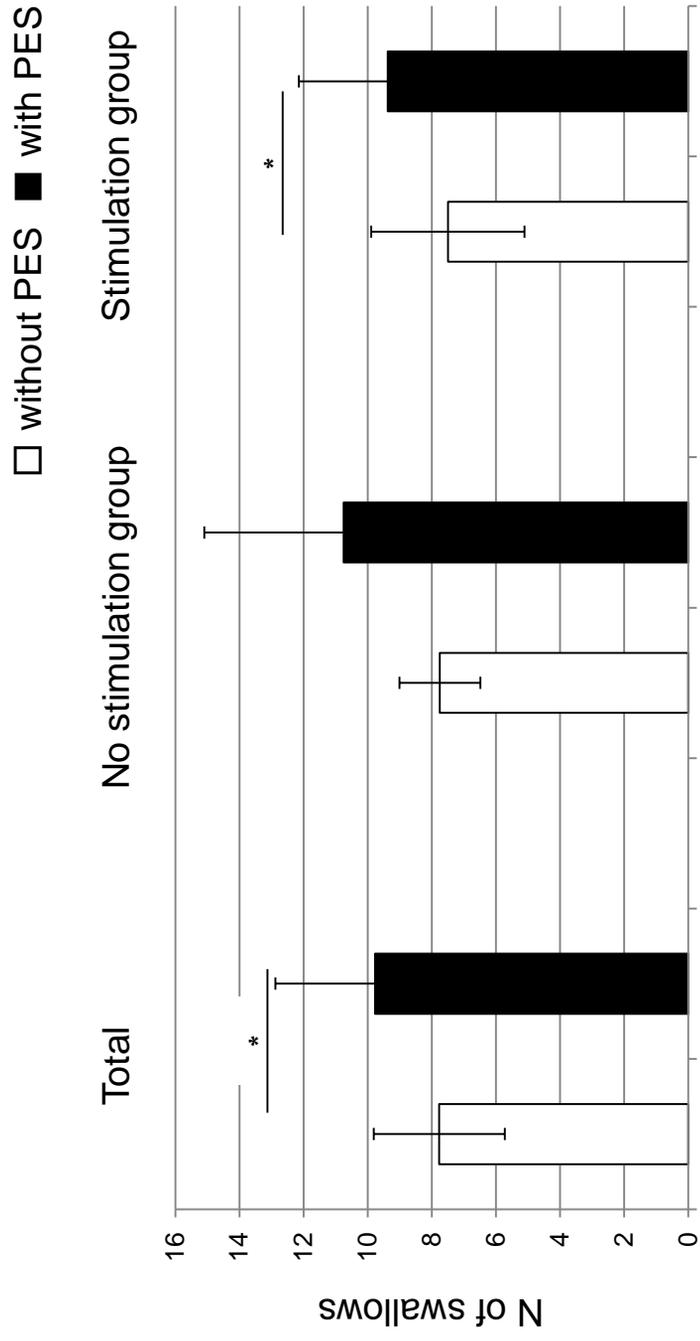


Fig. 4

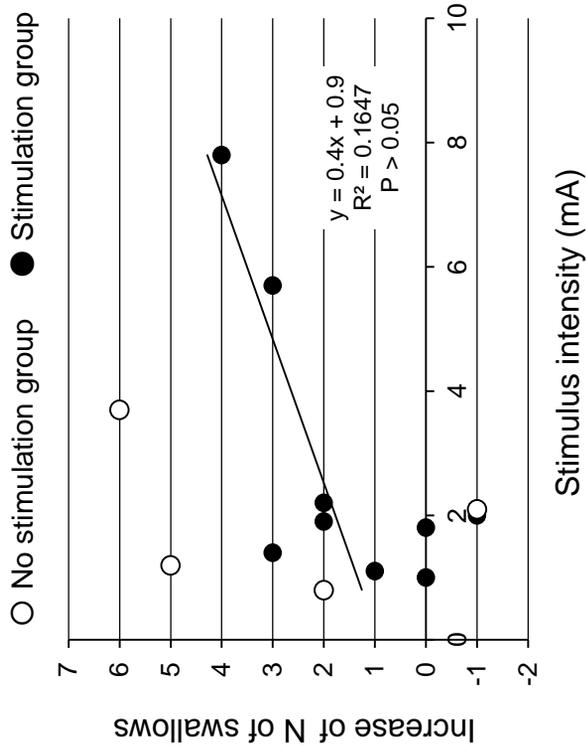


Fig. 5

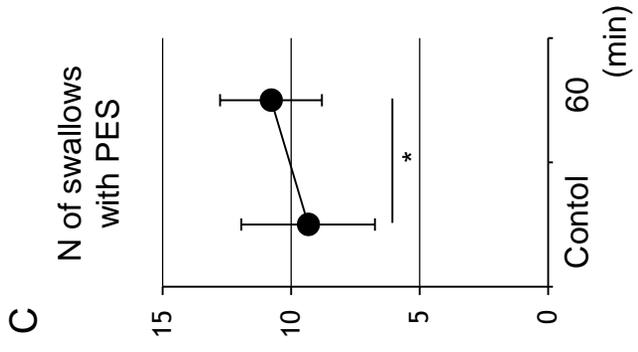
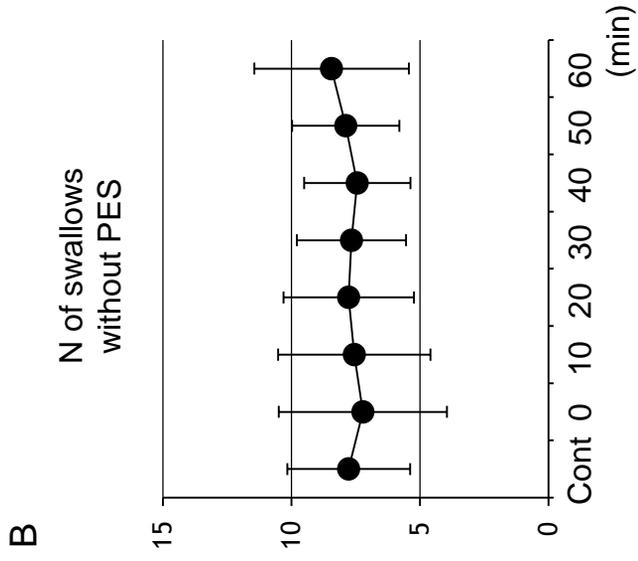
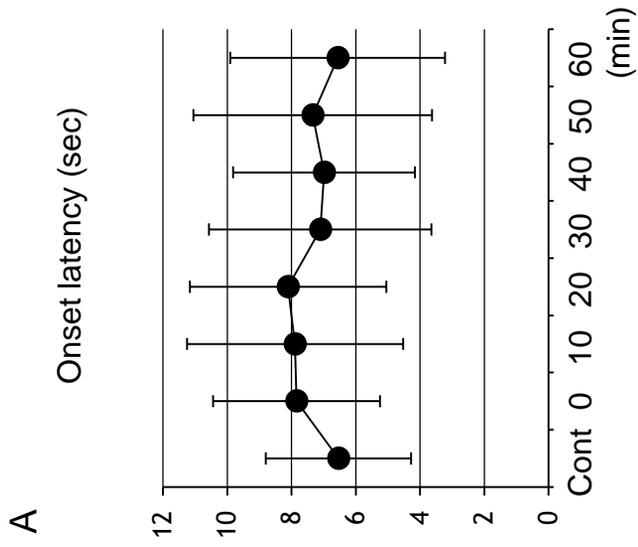
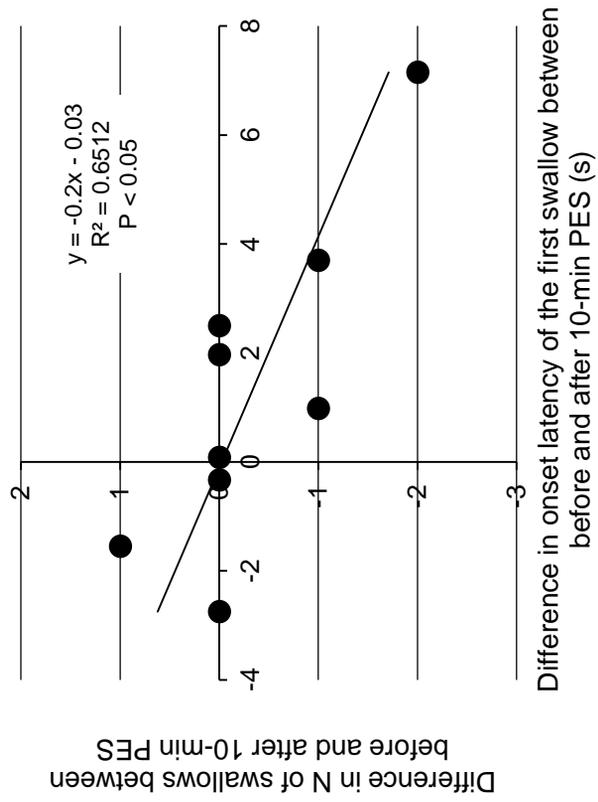


Fig. 6

A



B

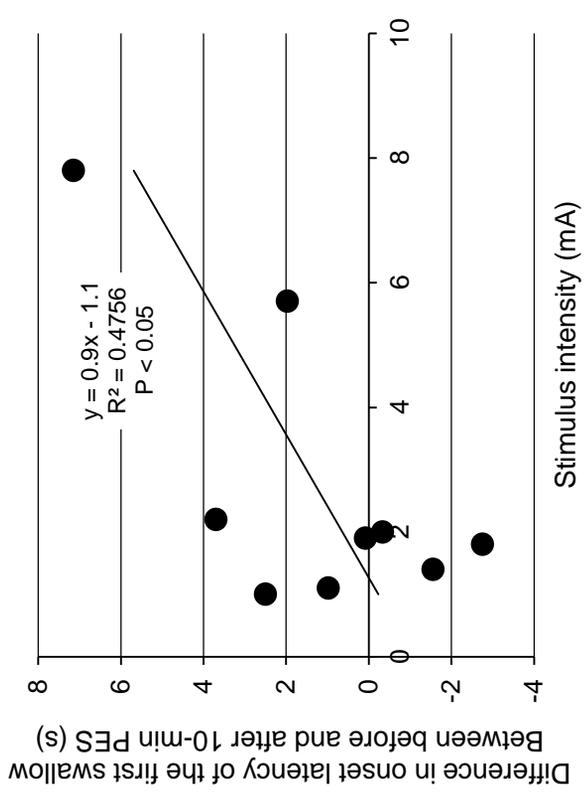


Fig. 7

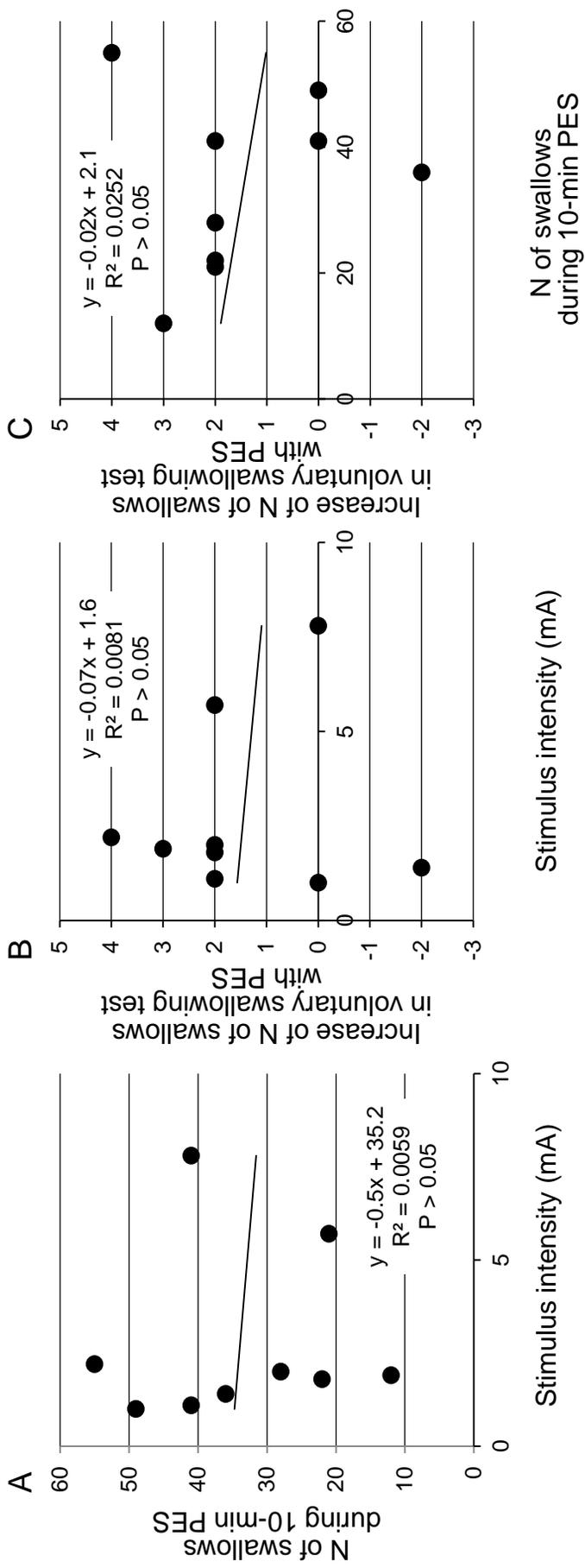


Fig. 8

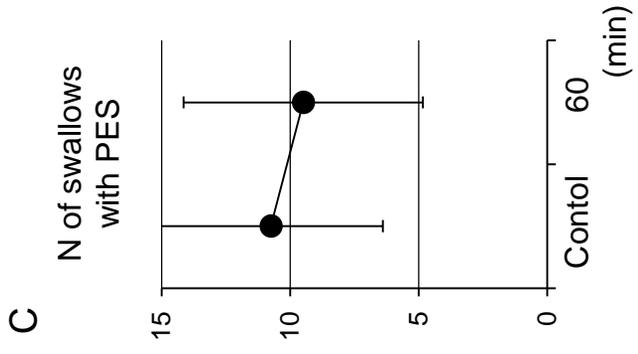
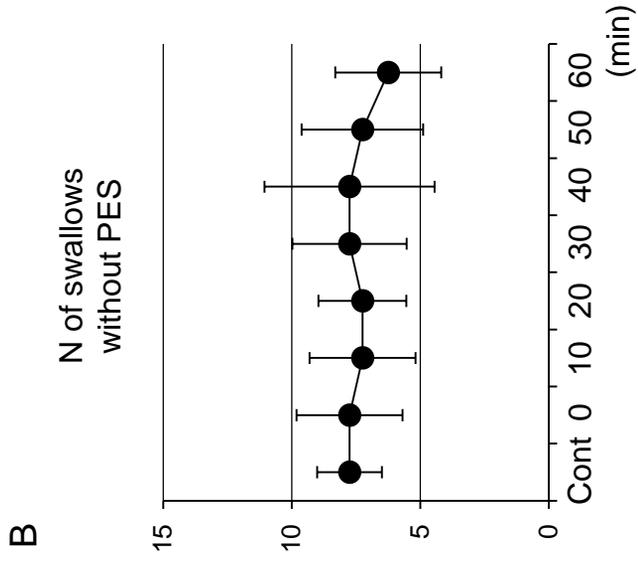
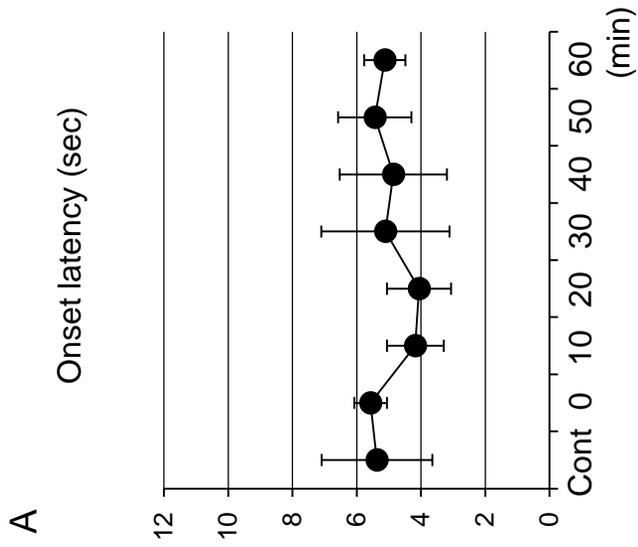


Fig. 9

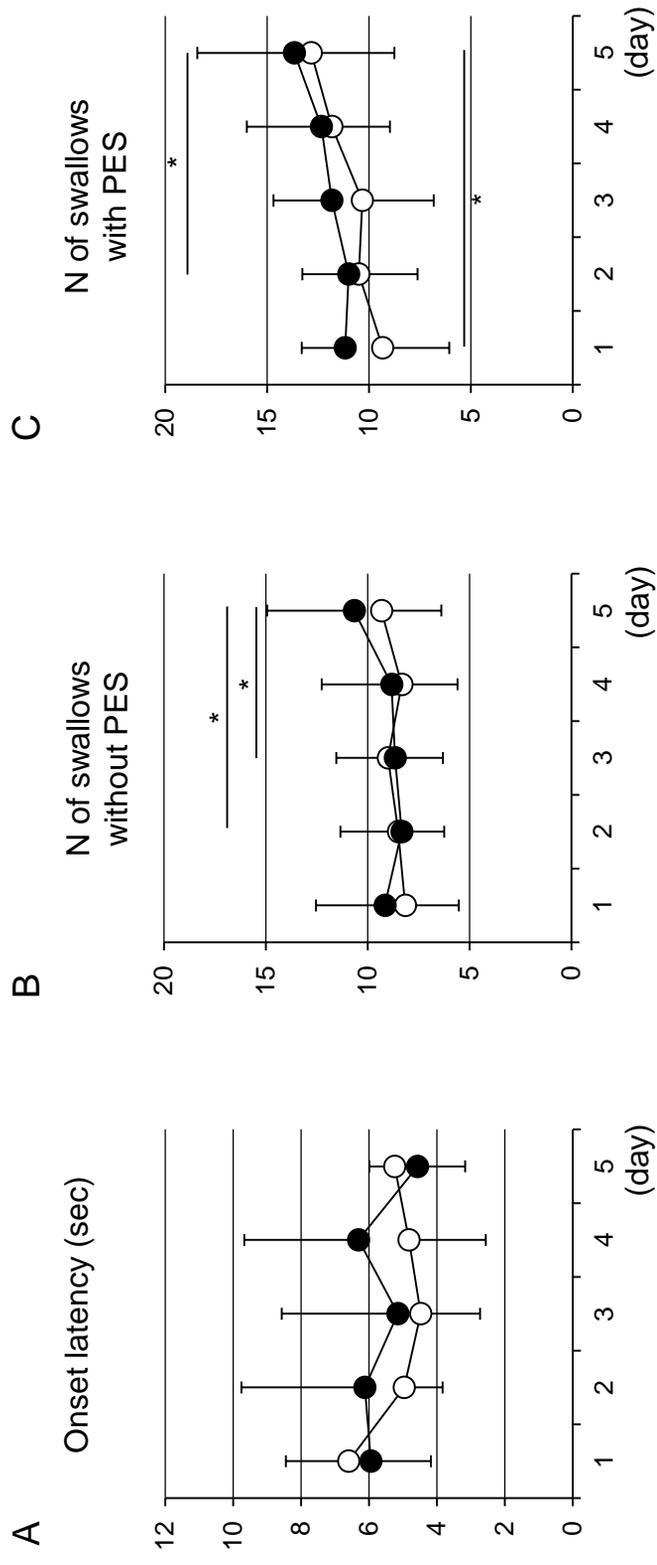


Fig. 10