Effect of carbonated water on swallowing performance in healthy volunteers

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Abstract

We investigated the effect of carbonated water application and swallowing of carbonated water on subsequent swallowing behavior in 12 healthy humans. We performed three experiments, involving 10 min of swallowing carbonated or natural water and 10 min of spitting carbonated water. Before and after each 10-min swallowing or spitting task, we evaluated involuntary and voluntary swallowing behaviors by measuring the onset latency of the first swallow after infusing natural water into the pharynx and by counting the number of voluntary swallows for 30 sec, respectively. We found that after 10 min of swallowing carbonated water, the onset latency gradually decreased over a 60-min period. Additionally, the electromyography (EMG) burst duration of suprahyoid muscles significantly decreased after 10 min of swallowing carbonated water. Immediately after 10 min of swallowing natural water, the number of voluntary swallows was significantly smaller compared with the control value. Our results suggest that 10 min of swallowing carbonated water produces a facilitatory effect on the excitability of the brainstem neural network involved in the swallowing reflex. Additionally, the facilitation of this pathway is associated with detrimental changes in swallowing behavior, specifically, shortening of the onset latency of the first involuntary swallow and shortened EMG duration.

Key words

Liquid stimulation, involuntary swallow, voluntary swallow, human

Introduction

Swallowing, an early stage of the eating process, involves complex sensorimotor neural components. The basic motor patterns involved in swallowing are programmed by the central pattern generator (CPG) in the medulla oblongata, and both the peripheral and central inputs into the CPG can trigger and/or modulate swallowing movements **(Jean 2001; Miller 1982)**. In other words, swallowing can be initiated either involuntarily or voluntarily in humans.

Peripherally evoked swallowing can be initiated by mechanical or chemical stimulation of the oropharynx or larynx. The 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. These regions are all innervated by the glossopharyngeal and vagal nerves **(Pommerenke 1928; Sinclair 1970; 1971; Storey 1968a; b)**. As initiation of the swallowing reflex is unaffected by ablation of the cortex, peripheral inputs may be sufficient to initiate swallowing within the neural circuit of the brainstem **(Tsuji *et al.* 2014)**.

Although sole stimulation applied to the trigeminal nerve innervating the oral region rarely evokes the swallowing reflex **(Jean 2001; Miller 1982)**, specific stimulation, such as noxious or taste stimulation, can modulate swallowing behaviors **(Babaei *et al.* 2010; Chee *et al.* 2005; Mistry *et al.* 2006; Tsujimura *et al.* 2009; Yahagi *et al.* 2008)**. The oral region contains rich sensory receptors, such as mechanoreceptors and taste receptors. Peripheral feedback from this region not only enters the brainstem network but also the cortex, where the motor pattern for any adaptation required to accommodate different peripheral inputs may be determined. Among the oral inputs that potentially modulate swallowing behaviors, the effect of carbonation has been extensively investigated in healthy humans and patients.

Plonk et al. **(Plonk *et al.* 2011)** stated that swallowing carbonated water did not change any swallowing parameters, such as electromyographic (EMG) activity or swallowing apnea in healthy humans. Michou et al. **(Michou *et al.* 2012)** investigated the effects of carbonation on swallowing behavior by recording normal, fast, and time-locked ('challenged') voluntary swallows. The authors found that participants were more successful in performing the challenged swallowing task with carbonated water compared with still water. They suggested that carbonation might affect higher centers involving swallowing. Miura et al. **(Miura *et al.* 2009)** reported that swallowing 60 mL of carbonated water increased the spectrum-integrated values of the total power components of suprahyoid EMGs by increasing the high frequency content. Some clinical reports have also shown that carbonated water has a modulatory effect on swallowing behaviors in neurologically impaired patients. Sdravou et al. **(Sdravou *et al.* 2012)** and Bulow et al. **(Bulow *et al.* 2003)** demonstrated that swallowing carbonated compared with noncarbonated liquid significantly decreased the penetration and/or aspiration of the bolus. Thus, most relevant studies have examined the immediate effect of carbonated water on swallowing performance, with the exception of one study by Morishita et al. **(Morishita *et al.* 2014)** demonstrating that swallowing a carbonated beverage affected the subsequent swallowing of water. However, the mechanisms underlying the immediate and persistent effects of carbonated water on swallowing behavior have not yet been clarified.

Peripheral stimulation can have a long-lasting effect on swallowing behavior. For instance, Fraser et al. **(Fraser *et al.* 2002)** and Power et al. **(Power *et al.* 2004)** showed that after 10 min of pharyngeal and oral electrical stimulation, corticobulbar excitability (as evaluated by motor evoked potentials) in the pharyngeal muscles increased, respectively. Based on these data, carbonated water applied to the oral cavity or pharynx is likely to have

not only an immediate effect, but also a long-term facilitatory effect on swallowing function. Accordingly, the aim of the present study was to clarify the effect of carbonated water application and swallowing of carbonated water on subsequent swallowing behaviors in healthy humans.

Materials and methods

Participants

Twelve healthy volunteers (7 males, mean age ± SD: 29.3 ± 3.3 years; age range: 25–36 years) participated in the study. All participants gave written informed consent, and no subject had a history of alimentary disease, 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-R49-03-21).

Physiological recordings

To identify and evaluate swallowing performance, we recorded EMG and electroglottographic (EGG) activities. We used similar methods as in our previous studies **(Aida *et al.* 2015; Nakamura *et al.* 2013)**. In brief, we attached bipolar surface EMG electrodes (ZB-150H; Nihon Kohden, Tokyo, Japan) to the skin over the anterior surface of the digastric muscle on the left side. EMG signals were detected in the suprahyoid muscle group, and filtered and amplified (low cut, 30 Hz and high cut, 2 KHz) (WEB-1000; Nihon Kohden, Tokyo, Japan) to remove movement-related artifacts. Bipolar surface EGG electrodes were positioned on the thyroid cartilage and the signals were amplified (EGG-D200; Laryngograph, London, UK). As with the EMG and EGG signals, we recorded button-pressing behavior. In the experimental procedure, the subject was instructed to hold

a button in his hand and press it with each swallow. The amplified EMG, EGG, and button pressing data were passed through an interface board (PowerLab; ADInstruments, Colorado Springs, CO, USA) and stored 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).

Stimulus solution

To evaluate the effect of chemical stimulation on swallowing performance, we prepared two stimulant solutions: carbonated water (Wilkinson Tansan; Asahi soft drinks, Tokyo, Japan) and natural water (Oishi-mizu; Asahi soft drinks, Tokyo, Japan). The carbonation level of the carbonated water was initially 3.68 v/v but had decreased to 2.22 v/v 10 min after opening the bottle cap (pH4.33-4.36). These solutions were kept at 19–27 °C, that is, close to room temperature.

Involuntary and voluntary swallowing tests

We conducted two swallowing tests to evaluate baseline swallowing performance in the participants: an involuntary and voluntary swallowing test. For the involuntary swallowing test, a thin tube with an outer diameter of 2.7 mm (NIPRO, Osaka, Japan) was first inserted into the midpharynx transnasally. We used videoendoscopy to confirm that the tip of the tube was positioned just above the epiglottic vallecula on the posterior wall of the midpharynx. Prior to recording in each test, the subject was asked to swallow his own saliva to clear the oral and/or pharyngeal cavity and then to rest and remain quiet with his eyes closed. Natural water (Oishi-mizu; Asahi Soft Drinks, Tokyo, Japan) was delivered through the tube using an infusion pump (KDS-100, Muromachi, Tokyo, Japan). To minimize the mechanical effect of the infused solution, we conducted the infusion at a very slow rate (0.1 mL/sec) until the first involuntary swallow had been evoked **(Fig. 1A)**.

As with the involuntary swallowing test, the subject was asked to swallow his own saliva before recording in the voluntary swallowing test. He was then instructed to engage in repetitive swallowing behavior as quickly as possible for 30 sec **(Fig. 1B)**.

Data collection

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. The current experiment comprised three tasks: 10 min of swallowing either carbonated or natural water and 10 min of spitting carbonated water **(Fig. 2)**. Of the 12 subjects, 12 and 10 subjects participated in the 10-min swallowing experiments and the 10-min spitting experiment, respectively. To reduce the effect of circadian variation or the environment on swallowing performance, the three experiments were performed at the same time of day for each individual in an air-conditioned room, with a room temperature of 20–24 °C and humidity of 40–70%. The order of the experiments was randomly determined.

After conducting the involuntary and voluntary swallowing tests to record baseline swallowing performance, we asked the participants to perform the experimental task. In the 10-min swallowing experiment, a small cup filled with 5 mL of either the carbonated or natural water was placed in front of the subject. The experimenter made a cue indicating that the participant should swallow the entire solution. This was repeated every 10 sec for 10 min, such that each subject swallowed a total of 300 mL in one experiment. Immediately after the 10-min swallowing task, we evaluated ongoing changes in swallowing by repeating the involuntary and voluntary swallowing tests every 10 min for 60 min.

In the 10-min spitting experiment, we only used carbonated water as a stimulus solution. The experimental setting was the same as in the 10-min swallowing experiment.

We first conducted involuntary and voluntary swallowing tests to evaluate baseline swallowing behavior. At an interval of 2 min, the subject was instructed to take 5 mL of carbonated water into the oral cavity and immediately spit it into a basin. This was repeated every 10 sec for 10 min. The involuntary and voluntary swallowing tests were repeated immediately after the 10-min spitting test every 10 min for 60 min.

Data analysis

EMG waveforms were full-wave rectified and smoothed (the time constant was 20 ms). We used the mean value (± SD) of a 5-sec EMG segment recorded at rest as a control. When data values exceeded the control by more than 2 SDs, the EMG burst was considered to be active **(Nakamura *et al.* 2013)**. Swallowing events could be detected from the EMG bursts, EGG bursts, and button pressing behavior. The time point of each swallow was defined as the peak of the associated EMG burst.

For the involuntary swallowing test, we measured the onset latency of the first involuntary swallow by calculating the time interval between the start of the infusion and the peak of the EMG burst associated with the first swallow **(Fig. 1A)**. To examine the effect of solution on the pattern of involuntary swallowing, we measured the duration, area, and peak amplitude of the EMG burst associated with the swallow. For the voluntary swallowing test, we counted the number of voluntary swallows in 30 sec. In addition, we calculated the time intervals between the first and second, second and third, and third and fourth consecutive swallows, and obtained an average of the time intervals in each trial for each individual.

Finally, we compared the mean values among the different times using one-way repeated-measures analysis of variance and Dunnett's test. Tests for statistical differences and comparison tests were performed using statistical software (SigmaPlot 12; Systat

Software Inc., San Jose, CA, USA). Statistical significance was set at $P < 0.05$. All values are expressed as mean ± SD (n=12 for swallowing test and n=10 for spitting test).

Results

Twelve participants completed the 10-min swallowing experiment, and 10 participants completed the 10-min spitting experiment. No subjects reported discomfort.

Effect of 10-min swallowing and spitting on involuntary swallowing performance

Immediately after 10 min of swallowing carbonated or natural water, the onset latency of the first involuntary swallow tended to be longer than that of the control, followed by a gradual decrease over the 60 min period **(Fig. 3A and B)**. This difference was significant only after 10 min of swallowing carbonated water. Involuntary swallowing initiation had been facilitated compared with the control value at 60 min after 10 min of swallowing carbonated water. After 10-min spitting of carbonated water, we found no significant difference in the onset latency of swallowing, although this value also tended to be longer immediately after the 10-min spitting task **(Fig. 3C)**.

We compared the EMGs associated with the first involuntary swallow among the control task, 0 min, and 60 min after the 10-min swallowing and spitting tasks **(Fig. 4)**. The duration of the EMG bursts was significantly shorter immediately and 60 min after 10 min of swallowing carbonated water compared with the control value. This was not the case after 10 min of swallowing natural water or spitting carbonated water, as we found no significant differences among the durations of the EMG bursts. We did not observe any significant differences among conditions in the other parameters such as area and peak.

Effect of 10-min swallowing and spitting on voluntary swallowing performance

We compared the number of voluntary swallows among the control and other conditions **(Fig. 5).** We also compared the time interval between the swallows among the control value,

at 0 min, and at 60 min **(Fig. 6)**. The number of voluntary swallows after 10 min of swallowing and spitting carbonated water did not change significantly throughout the recording periods **(Fig. 5A and C)**. On the other hand, the number of voluntary swallows significantly decreased immediately after 10 min of swallowing natural water **(Fig. 5B)**. Regarding the time interval between the swallows, we found a significant difference between the control and 0 min value after 10 min of swallowing natural water only **(Fig. 6B)**. Otherwise, we found no significant differences among the conditions **(Fig. 6A and C)**.

Discussion

In the present study, we evaluated the effect of 10 min of stimulation with either natural or carbonated water on involuntary and voluntary swallowing performance. After 10 min of swallowing carbonated water, the onset latency of the first involuntary swallow gradually decreased over a 60-min period. Additionally, we found a significant difference between control values and those 60 min after 10 min of swallowing. The EMG burst duration significantly decreased after 10 min of swallowing carbonated water. This was not the case after 10 min of swallowing natural water or 10 min of spitting carbonated water, as we found no significant differences in the onset latency or EMG characteristics among these conditions. Immediately after 10 min of swallowing natural water, the number of voluntary swallows during a 30 sec period was significantly smaller and the time interval between the voluntary swallows was significantly larger compared with the control values. Compared with control values, we found no differences in the number of voluntary swallows or in the time interval between voluntary swallows after 10 of swallowing or spitting carbonated water. This indicates that the potential to initiate a voluntary swallow was not affected in these conditions. The possible mechanisms for this modulation are discussed in the following

section.

Methodological considerations

In the present study, we performed both involuntary and voluntary tests to evaluate swallowing function. In the former, natural water was applied to the midpharynx at a very slow rate (0.1 mL/sec). The mean onset latency of the first swallow in the control condition varied between approximately 3 and 6 sec, indicating that the estimated volume of infused water for the first swallow ranged between 0.3 and 0.6 mL. Rudney et al. **(Rudney *et al.* 1995)** estimated that the volume of saliva that can evoke a spontaneous natural swallow is 0.46 mL in a normal subject, suggesting that our involuntary swallowing test effectively mimicked natural spontaneous saliva swallowing.

Although we did not clarify whether a small volume ranging between 0.3–0.6 mL was large enough to stimulate the mechanoreceptors responsible for swallowing initiation, we presume that water-sensitive receptors in the pharynx were involved in swallowing initiation in the involuntary swallowing test **(Shingai 1977; Storey 1968a; b)**. Previous studies have demonstrated that pharyngeal application of distilled water with a small infusion rate can facilitate voluntary swallowing in humans **(Kitada *et al.* 2010; Nakamura *et al.* 2013; Yahagi *et al.* 2008)**. In addition, because initiation of the swallowing reflex is not interrupted after ablation of the cortex, peripheral inputs may be sufficient to initiate swallowing within the neural circuit of the brainstem **(Tsuji *et al.* 2014)**. Thus, the swallowing elicited in our study can most likely be regarded as a swallowing reflex evoked by peripheral afferents, although we cannot completely exclude the possibility that subliminal peripheral stimulation resulted in cerebral cortical activation **(Kern and Shaker 2002)**.

To evaluate voluntary swallowing performance, we counted the number of voluntary swallows and measured the time interval between the voluntary swallows.

Amongst clinicians, the former measurement is known as the repetitive saliva swallowing test (RSST), developed as a safe and simple screening test for dysphagia **(Tamura *et al.* 2002)**. However, this test is only used to identify functional dysphagia, and not to quantify function in healthy humans. The latter measurement was also used in a previous study **(Aida *et al.* 2015; Kitada *et al.* 2010)**. The time interval between swallows is a good predictor of the number of swallows in a certain time period, and, compared with the RSST, may be more useful in detecting small differences in the potentials required to initiate voluntary swallows **(Aida *et al.* 2015)**.

Possible factors involved in the modulation of swallowing behavior

In the present study, we evaluated modulations in involuntary and voluntary swallowing behaviors after 10 min of swallowing. First, we found that the onset latency of the first involuntary swallow deceased 60 min after 10 min of swallowing carbonated water. Second, the EMG duration significantly decreased after 10 min of swallowing carbonated water. Finally, the number of voluntary swallows decreased and time interval between the swallows increased immediately after 10 min of swallowing natural water. These findings lead us to question (1) whether 10 min of swallowing, that is, swallowing 60 times in 10 min, influenced the modulation of swallowing behaviors, and (2) whether carbonated water-sensitive fibers were involved in the modulation.

To address the first question, we compared the results for 10 min of swallowing natural water with those for 10 min of swallowing carbonated water. If swallowing every 10 sec for 10 min modulated involuntary swallowing initiation or EMG duration, then 10 min of swallowing natural water should cause similar changes. In the present study, however, we only observed a significant decrease in the EMG duration and onset latency of the first involuntary swallow after 10 min of swallowing carbonated water. Furthermore, the time

interval between the voluntary swallows was significantly larger immediately after 10 min of swallowing natural water compared with the control, suggesting that the difference might not be attributed to 10-min periods of repetitive swallowing.

Fraser et al. **(Fraser *et al.* 2003)** demonstrated that, immediately after 10 min of water swallowing, pharyngoesophageal craniobulbar and corticobulbar excitabilities increased. These results suggest that 10 min of repetitive swallowing had an immediate facilitatory effect on both involuntary and voluntary swallowing behaviors. However, our current data indicate that 10 min of swallowing had an inhibitory rather than a facilitatory effect **(Figs. 5B and 6B)**. It is likely that there is a critical difference between the methodologies used in their study and ours. Fraser et al. evaluated the excitability of the craniobulbar projection using transcranial magnetic stimulation of the supraorbital nerve, innervated by the trigeminal nerve. Tsuji et al. **(Tsuji *et al.* 2014)** showed that continuous superior laryngeal nerve (SLN) stimulation suppressed subsequent swallowing initiation in anesthetized rats. The authors also demonstrated that decerebration had no effect on the reduction of evoked swallows. This result leads us to suggest that reduced sensory afferent nerve firing and/or transsynaptic responses, as well as part of the brainstem neural network, were involved in the adaptation of the swallowing reflex or inhibition of the swallowing neural network within the brainstem. In fact, immediately after 10 min of swallowing either type of solution, the onset latency of the first involuntary swallow tended to be larger compared with the control. In the present study, to evaluate involuntary swallowing function represented by the onset latency of the first involuntary swallow, we evoked involuntary swallowing by injecting natural water into the pharynx, innervated by the glossopharyngeal or SLN. The effect of repetitive swallowing may be different between the trigeminal and glossopharyngeal/superior laryngeal afferent-pathways. An inhibitory trend was observed

immediately after 10 min of either spitting or swallowing carbonated water. Tsuji et al. **(Tsuji *et al.* 2014)** showed that subthreshold continuous SLN stimulation, which did not evoke swallowing, also suppressed subsequent swallowing initiation. The nucleus tractus solitarii (NTS) is a major brainstem site for initiation and modulation of the swallowing reflex **(Jean 2001)**. Previous studies have demonstrated that the NTS receives afferent inputs from the trigeminal nucleus **(Zerari-Mailly *et al.* 2005)**. It is possible that adaptation of the initiation of swallowing reflex occurred just after the 10-min application of carbonated water to the oral cavity, of which the mucosa region is innervated by the trigeminal nerve.

Although Fraser et al. **(Fraser *et al.* 2003)** reported an increase in pharyngolaryngeal corticobulbar excitability after 10 min of swallowing water, this does not indicate that there was an increase in the cortical excitability involved in the initiation of voluntary swallowing. In the current results, voluntary swallowing initiation was inhibited immediately after 10 min of swallowing natural water, but not after 10 min of swallowing carbonated water. We wish to consider another explanation for this observed difference between the conditions, that is, stimulus solutions (see below).

To address the second question, we compared the results between 10 min of swallowing and 10 min of spitting carbonated water. These conditions differed according to whether the subject swallowed the carbonated water, and whether the carbonated water was exposed only to the oral cavity or to both the oral cavity, pharynx and possibly esophagus and stomach during the 10-min period. Previous reports have investigated how oral and pharyngeal sensations are modulated by the application of carbonated water, both chemogenic and mechanical (bubbles of carbon dioxide). The use of a carbonic anhydrase blocker such as acetazolamide **(Dessirier *et al.* 2000; Graber and Kelleher 1988)** and dorzolamide **(Simons *et al.* 1999)** weakened oral irritant sensation in humans. Such

blockers significantly reduced the responses of nociceptive afferents in the lingual nerve **(Dessirier *et al.* 2000; Komai and Bryant 1993)** and of neural activity in the trigeminal nucleus **(Simons *et al.* 1999)** in animals. These findings suggest that carbonated water activates nociceptive fibers in the oral mucosa when dissolved carbon dioxide reacts with carbonic anhydrase in salivary enzymes and produces carbonic acid. The oral sensation evoked by carbonated water is mediated by capsaicin-sensitive nociceptors **(Dessirier *et al.* 2000; Dessirier *et al.* 2001)**. The transient receptor potential vanilloid-1 (TRPV1) receptor, which acts as a polymodal nociceptor activated by capsaicin, heat, and acid, are widely distributed through the foodway, including mouth and pharynx. Direct stimulation of such TRP channels on peripheral neurons activates the neurons by opening large-cation channels **(Dhaka *et al.* 2006)**. Arai et al. **(Arai *et al.* 2010)** tested the responses of TRPV1 to acids applied to the mouth, pharynx, and larynx. The authors found that in the glossopharyngeal nerve and SLN only, iodo-resiniferatoxin (I-RTX), a potent TRPV1 antagonist, significantly suppressed responses to acids. This suggests that TRPV1 receptors are important for responding to acid stimulation not only in the mouth but also in the pharynx. Acid-sensing ion channels (ASICs), which belong to the epithelial sodium channel/degenerin superfamily, have also been identified in the central and peripheral nervous system **(Alvarez de la Rosa *et al.* 2003; Alvarez de la Rosa *et al.* 2002; Holzer 2009; Price *et al.* 2001)**. The expression of ASIC proteins has been frequently noted in dorsal root ganglion and trigeminal ganglion neurons **(Alvarez de la Rosa *et al.* 2002; Chen *et al.* 1998; Garcia-Anoveros *et al.* 1997; Molliver *et al.* 2005; Waldmann *et al.* 1997)**. It is possible that in the present study, the TRPV1 in the pharynx, which would have reacted to the 10-min exposure to carbonated water, might have modulated swallowing behaviors.

There was a remarkable difference in the EMG duration of involuntary swallowing between carbonated and natural water. Specifically, this duration significantly decreased after 10 min of carbonated water swallowing. Ertekin and his colleague **(Ertekin and Aydogdu 2003)** suggested that conditions with fewer sensory inputs may cause a delay in the initiation of the swallowing reflex, and a prolongation of EMG duration. This was not the case in the present study because the difference in the peripheral circumstances was small between the situations: the estimated volume of water per swallow ranged from 0.3 to 0.6 mL in the involuntary swallowing test. Therefore, 10 min of swallowing carbonated water might cause an increase in the brainstem excitability involved in swallowing initiation. This could be explained by our finding that the number of voluntary swallows and the time interval between the voluntary swallows did not change after 10 min of swallowing carbonated water, but decreased after 10 min of swallowing natural water. Perhaps the increased excitability of the neural network activated by carbonated water application (swallowing) compensated for the adaptation or inhibition of the swallowing neural network, at least within the brainstem.

Possible mechanisms of the long-term effect of carbonated water application on swallowing behaviors

In the present study, we observed a long-term effect of 10 min of swallowing carbonated water only in the involuntary swallowing condition. Specifically, the onset latency of the first swallow was significantly shorter 60 min after 10 min of swallowing, and the EMG duration significantly decreased although the number of voluntary swallows and the time interval between the swallows did not change. Previous human studies have shown that oral **(Power *et al.* 2004)** and pharyngeal electrical stimulation **(Fraser *et al.* 2002; Hamdy *et al.* 1998)** can produce a unique long-term effect on corticobulbar excitability. Specifically, 10 min of stimulation increased pharyngeal/esophageal cortical excitability for at least 30 min

afterwards. Although these findings suggest that the involvement of the corticobulbar projection contributed to the modulation of swallowing behaviors after 10 min of stimulation, the parameters used by those authors were not identical to ours.

It is widely accepted that oral health care can reduce the incidence of fever and pneumonia and the oropharyngeal bacteria count in elderly people **(Adachi *et al.* 2002; Ishikawa *et al.* 2008; Yoneyama *et al.* 1999; Yoneyama *et al.* 2002)**. Ebihara and his colleagues showed that intensive oral care improved dysphagia and suggested that the improvement was possibly due to restoring the swallowing and cough reflexes in such populations **(Watando *et al.* 2004; Yoshino *et al.* 2001)**. Yoshino et al. **(Yoshino *et al.* 2001)** showed that salivary Substance P concentration was elevated after 1 month of intensive oral care. Because Substance P is reported to play a major role in the initiation of both cough and swallowing reflexes **(Ebihara *et al.* 1993; Sekizawa *et al.* 1996)**, one can expect that the improvement of those reflexes could be attributed to the elevation of Substance P levels. Capsaicin has been shown to release substance P from the end of the unmyelinated sensory nerve on the upper respiratory tract **(Ebihara *et al.* 2011)**. Daily sensory stimulation with capsaicin promoted beneficial changes in neuroplasticity and swallowing function **(Ebihara *et al.* 2005)**. Taken together, our data indicate that a 10-min application of carbonated water might have a long-term effect on the swallowing reflex, in that capsaicin-sensing fibers might be activated and the neural network in the lower brainstem involved in swallowing would be provoked by the release of Substance P. We hope to precisely clarify the mechanisms underlying the long lasting facilitatory effect of 10 min of swallowing carbonated water in future research.

In conclusion, we have shown that 10 min of swallowing carbonated water produces a facilitatory effect on the excitability of the brainstem neural network involved in

the swallowing reflex. Additionally, the facilitation of this pathway appears to be associated with detrimental changes to swallowing behavior, such as a shorter onset latency of the first involuntary swallow and shorter EMG duration. These results may provide ideas for future therapeutic strategies.

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Figure captions

Fig. 1. Examples of EGG and EMG recordings obtained in the involuntary (A) and voluntary (B) swallowing tests. The data were obtained from one subject on the same day. A: Dashed lines marked by a and b indicate the start of the water infusion and the peak of the EMG bursts during swallowing, respectively. In this example, which was conducted before a 10-min swallowing experiment, the onset latency of the first swallow was 2.81 sec. Prior to water infusion, the subject was instructed to perform voluntary swallowing (shown by arrow). B: Dashed lines indicate the start and end of recording period (30 sec). In this example, which was conducted before a 10-min swallowing experiment, the number of voluntary swallows was 12. As with the involuntary swallowing test, the subject was instructed to perform voluntary swallowing before the water infusion (shown by arrow). fEMG, filtered (rectified and smoothed) EMG.

Fig. 2. Experimental study protocol. We conducted involuntary and voluntary swallowing tests followed by 10 min of swallowing or 10 min of spitting. Immediately afterwards and every 10 min up to 60 min, we conducted both the involuntary and voluntary swallowing tests.

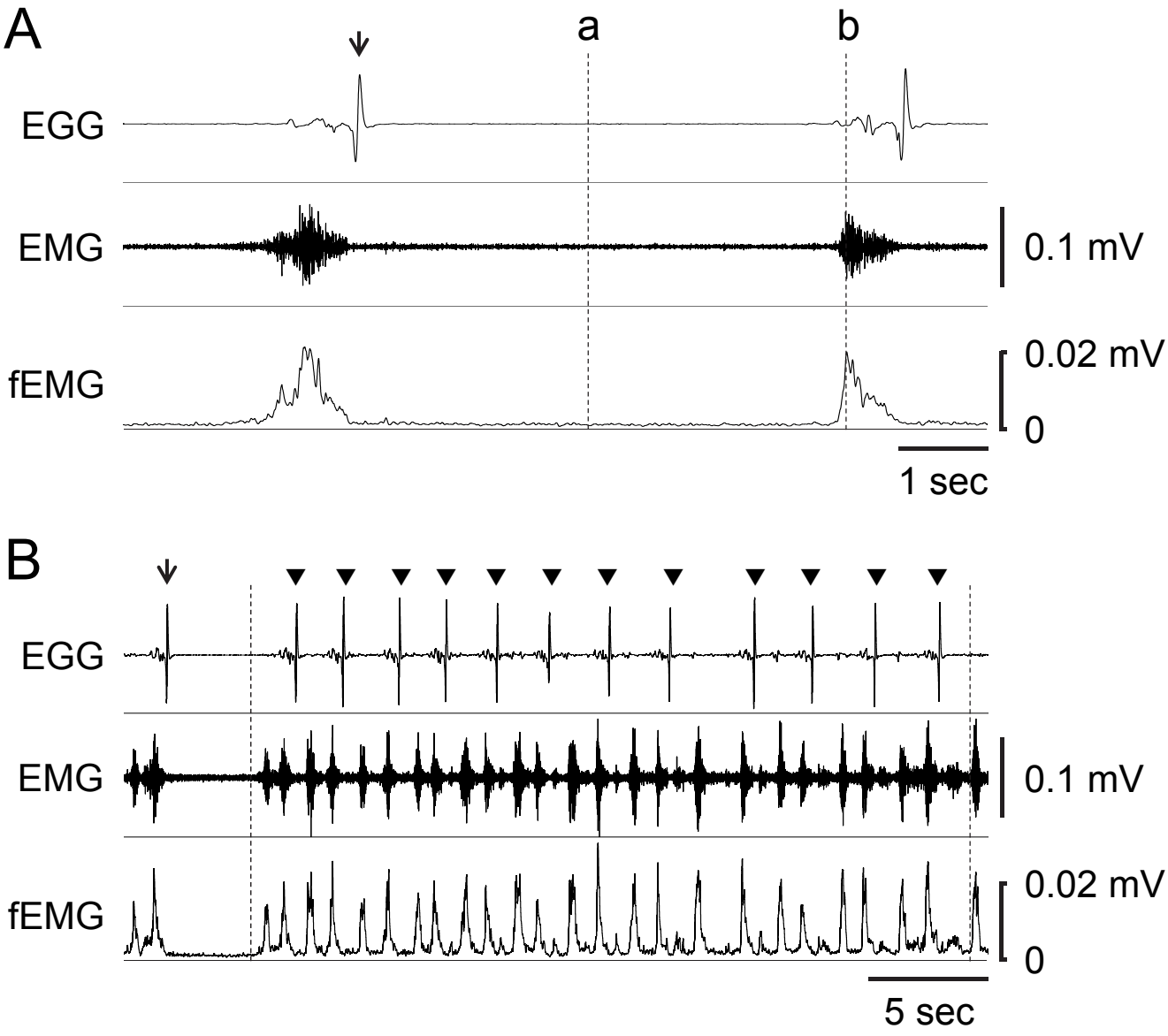
Fig. 3. Effect of 10 min of swallowing or spitting on subsequent involuntary swallowing. A: Following 10 min of swallowing carbonated water, we found a significant difference in the onset latency of the first swallow between the control (c) and 60 min after the task. Although the onset latency of the first swallow tended to be larger immediately after 10 min of swallowing, this difference was not significant. B and C: The onset latency of the first

swallow tended to be larger immediately after 10 min of swallowing natural water (B) and 10 min of spitting carbonated water (C) compared with the control. We observed a return to baseline within 60 min. We found no significant differences among the measured time points. *P<0.05.

Fig. 4. Effect of 10 min of swallowing and spitting on EMG burst characteristics in the involuntary swallowing test. A: Following 10 min of swallowing carbonated water, we found significant differences in the burst duration between the control (c), immediately after the task, and 60 min after the task. ***P<0.001. We found no significant differences in terms of area or peak among the measured time points. B and C: Following 10 min of water swallowing (B) and 10 min of carbonated water spitting (C), we found no significant differences in the variables among the measured time points.

Fig. 5. Effect of 10 min of swallowing and spitting on the subsequent number of voluntary swallows.
Following 10 min of swallowing carbonated (A) and natural (B) water and 10 min of spitting carbonated water (C), we found no significant differences in the number of swallows among the measured time points. c, control. *P<0.05.

Fig. 6. Effect of 10 min of swallowing carbonated (A) and natural (B) water and 10 min of spitting carbonated water (C) on the time intervals between two consecutive voluntary swallows in the voluntary swallowing test. We found a significant difference between the control (c) and immediately after 10 min of swallowing natural water only.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a
b
EGG
EMG
fEMG
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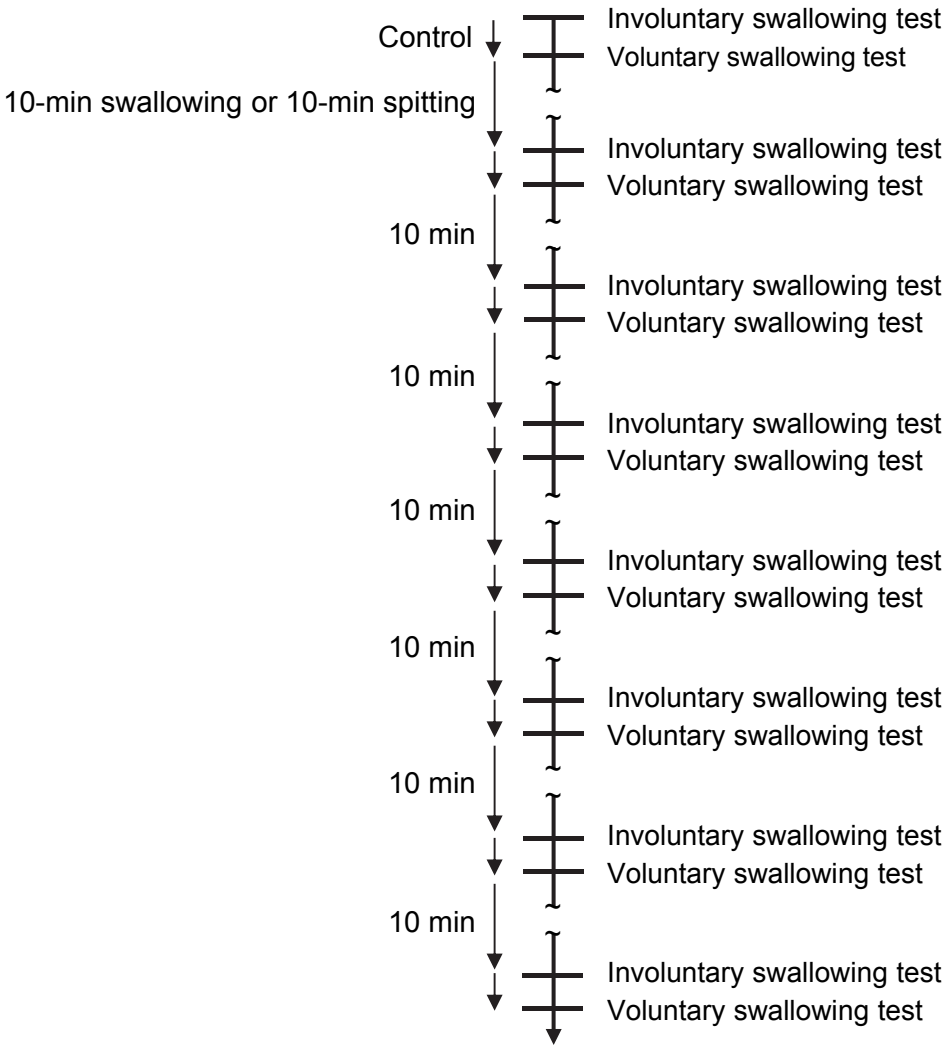
0.02 mV
0
1 sec
B
EGG
EMG
fEMG
0.1 mV
0.02 mV
0
5 sec

Fig. 1


Control
10-min swallowing or 10-min spitting
10 min
10 min
10 min
10 min
10 min
Involuntary swallowing test
Voluntary swallowing test
Involuntary swallowing test
Voluntary swallowing test
Involuntary swallowing test
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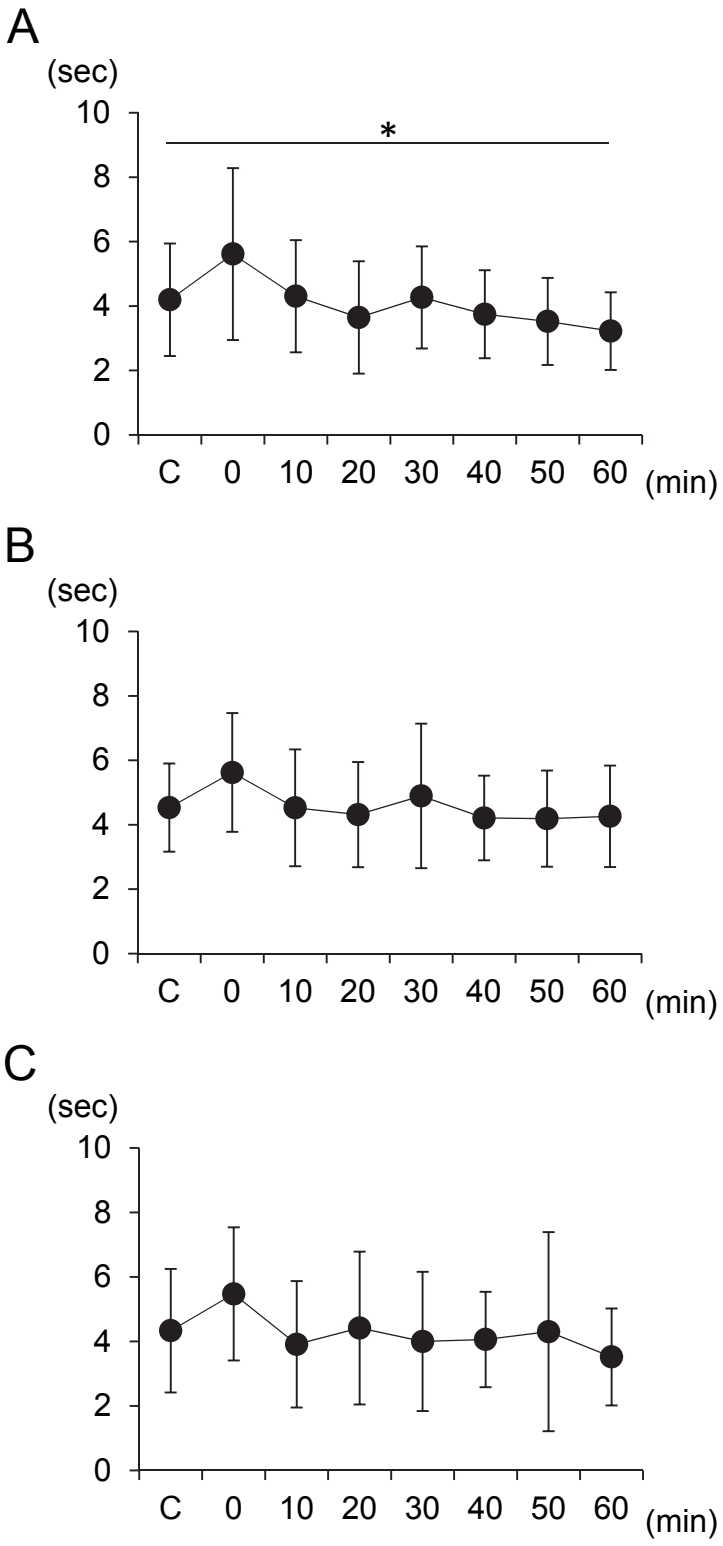
Voluntary swallowing test

Fig. 2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8
6
4
2
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*
C
0
10
20
30
40
50
60
(min)
B
(sec)
10
8
6
4
2
0
C
0
10
20
30
40
50
60
(min)
C
(sec)
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8
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2
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C
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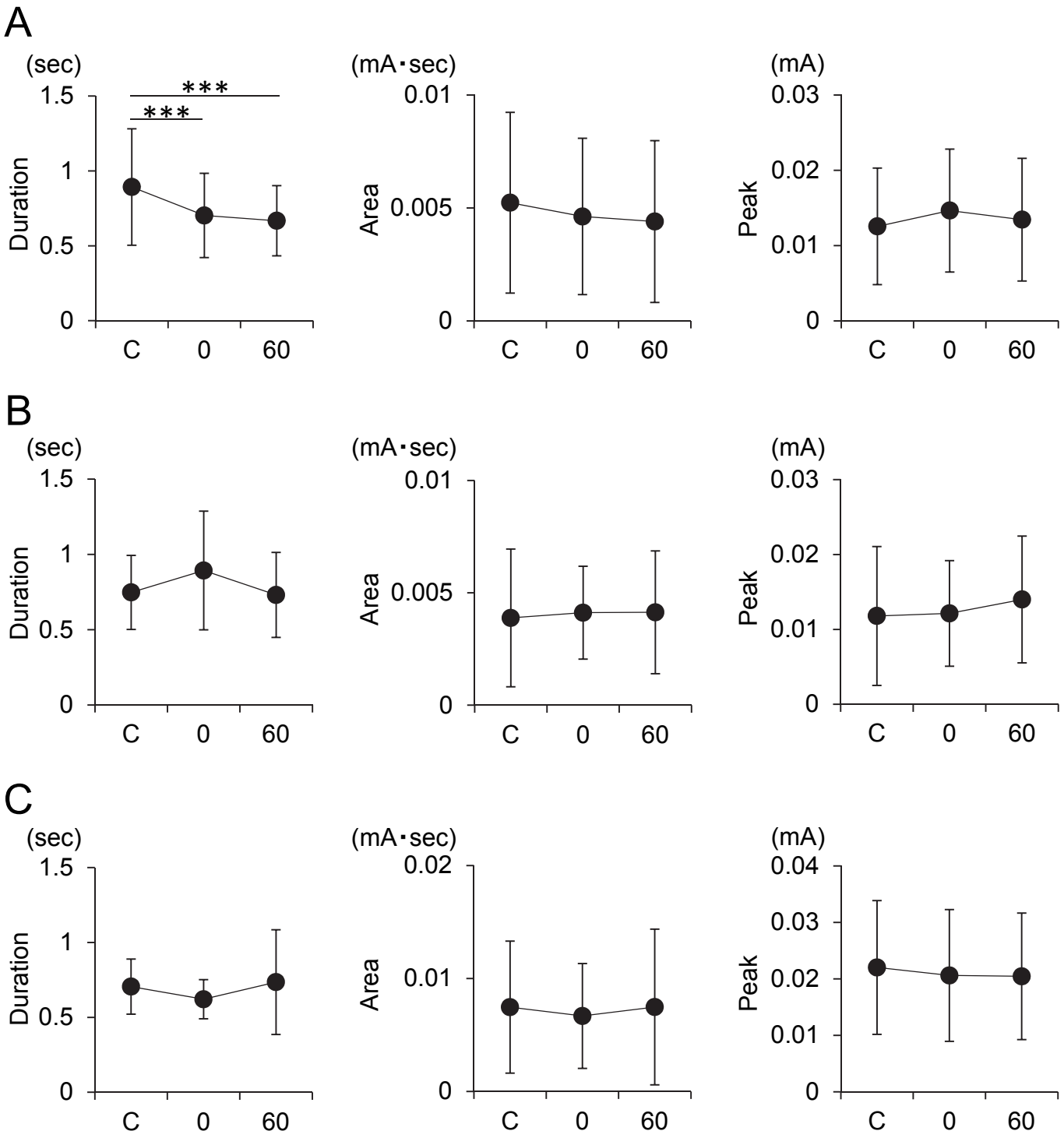
40
50
60
(min)

Fig. 3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0
60
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0.03
0.02
0.01
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Peak
C
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B
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1.5
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Area
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0.02
0.01
0
Peak
C
0
60
C
(sec)
1.5
1
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Duration
C
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60
(mA・sec)
0.02
0.01
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0.01
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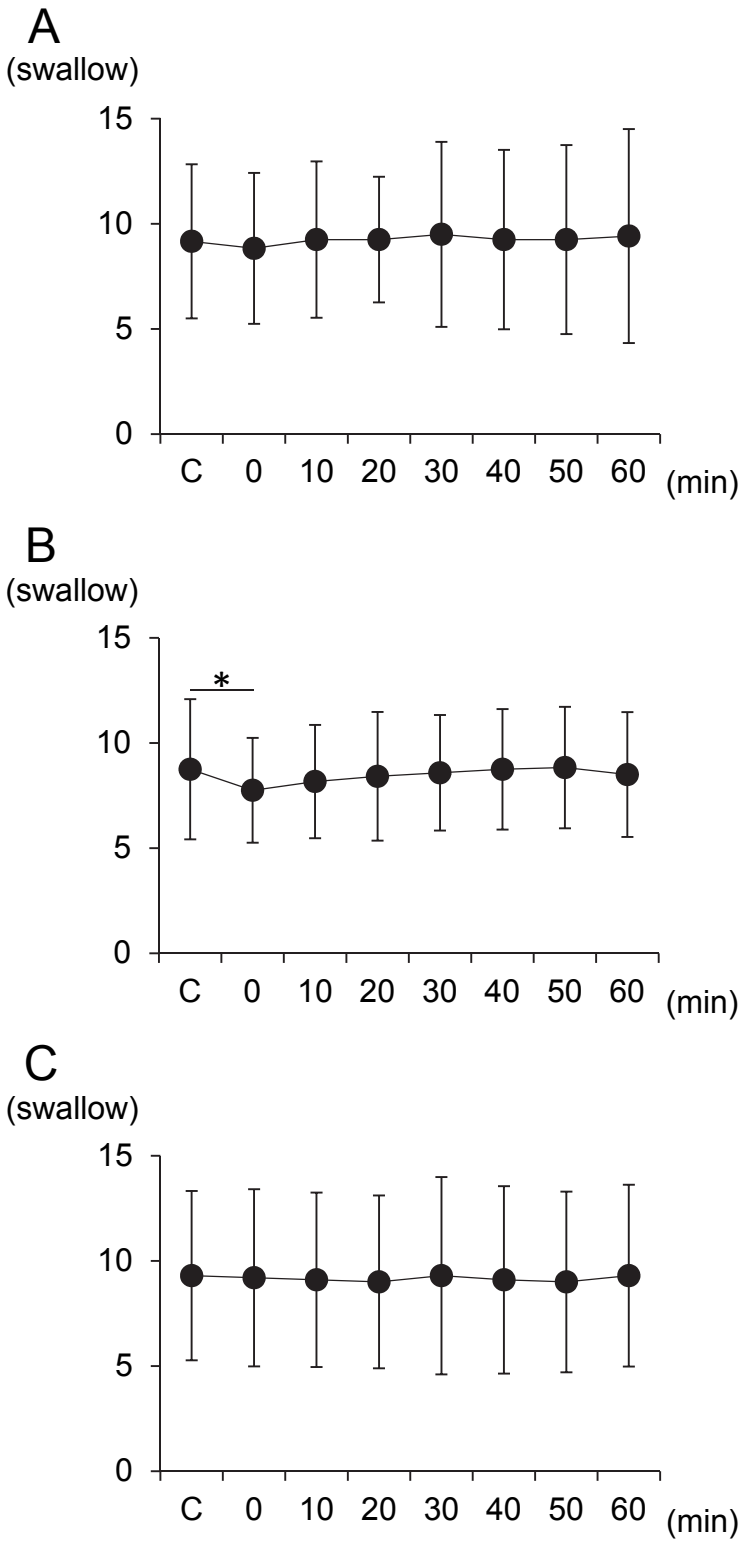
Peak
C
0
60

Fig. 4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10
5
0
C
0
10
20
30
40
50
60
(min)
B
(swallow)
15
10
5
0
*
C
0
10
20
30
40
50
60
(min)
C
(swallow)
15
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5
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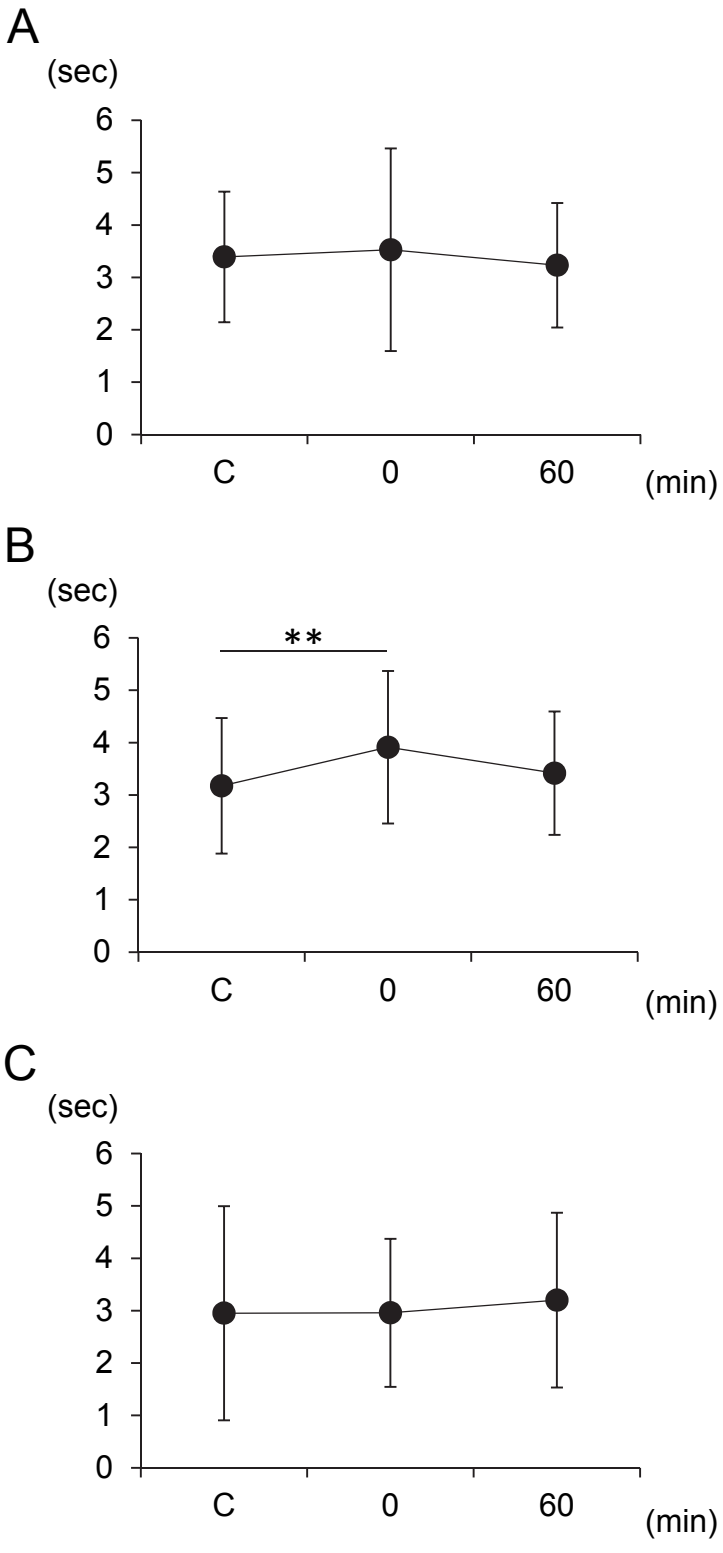
40
50
60
(min)

Fig. 5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(sec)
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3
2
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C
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5
4
3
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C
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(min)

Fig. 6