

Effects of intra-esophageal acid infusion and a stress task on masseter muscle activity and autonomic nervous activity in wakefulness

Short running title: Factors effecting masseter muscle activity

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ABSTRACT

Background: Gastroesophageal reflux disease may be an important risk factor for awake bruxism. Additionally, it has been known that a psychological stress task affects masseter muscle activity, and autonomic nervous system (ANS) activity modulation induces masseter muscle activity.

Objectives: This study aimed to investigate whether task-induced stress and experimental esophageal acid infusion increase masseter muscle activity and alter ANS activity, compared to rest task and esophageal saline infusion, respectively.

Methods: Polygraphic monitoring, consisting of electromyography of the masseter muscle and electrocardiography was performed in 12 healthy adult men during 30-min interventions with intra-esophageal saline or acid infusion, while reading a book quietly, as rest, and while performing calculation, as a stress task.

Results: At rest, masseter muscle activity and parasympathetic nervous system (PNS) activity during acid infusion was significantly higher ($P=0.019$) and lower ($P=0.021$) than during saline infusion, respectively. During saline infusion, both masseter muscle activity and sympathetic nervous system (SNS) activity or PNS activity while performing the calculation task were higher ($P = 0.022$ and 0.012 , respectively) or lower ($P = 0.007$) than those during the reading task, respectively. In two-way repeated-measures ANOVA, intra-esophageal infusion (saline or acid) significantly affected masseter muscle activity ($P=0.008$) and PNS activity ($P=0.021$). However,

performing tasks (reading or calculation) significantly affected only PNS activity ($P=0.028$).

Conclusion: Intra-esophageal acid infusion significantly increased masseter muscle activity and decreased PNS activity. In contrast, stress task significantly decreased PNS activity, but only modestly increased masseter muscle activity and SNS activity.

Key words: awake bruxism, gastroesophageal reflux disease, acid infusion, psychological stress, sympathetic nervous system, parasympathetic nervous system

BACKGROUND

Awake bruxism (AB) is a masticatory muscle activity occurring during wakefulness and is characterized by repetitive or sustained tooth contact and/or by bracing or thrusting of the mandible.¹ According to recent reports, gastroesophageal reflux (GER) disease (GERD) may be a risk factor for AB and sleep bruxism in adults.²⁻⁵ Intra-esophageal acid stimulation, which is a model of GER, has been reported to induce swallowing and increased masseter muscle activity and change ANS activity under wakeful conditions.⁶

AB is thought to be associated with stress caused by family responsibilities or work pressure.⁷⁻¹⁰ However, these associations are not based on strong evidence,¹¹ and whether a relationship exists between psychological stress and bruxism or increase of masseter muscle activity under wakeful conditions remains unclear. Additionally, to evaluate masseter muscle activity under wakeful conditions, what participants are doing is important. For example, the type of ability task (i.e., playing a videogame) has been reported to affect the occurrence of clenching episodes and muscular activity.¹² An ability task, such as calculation, is known to induce psychological stress^{13,14}; such psychological stress tasks affect ANS activity. Thus, we hypothesized that psychological stress tasks increase masseter muscle activity and alter ANS activity, as does intra-esophageal acid stimulation, which has been reported to change ANS activity and is related to masseter muscle activity during wakefulness.⁶ Additionally, it

is unclear whether esophagus acid stimulation and psychological stress task have an effect on these activities in awake individuals.

This experimental study aimed to investigate whether calculation task-induced psychological stress and experimental esophageal acid infusion increase masseter muscle activity and alter ANS activity, compared to rest task and esophageal saline infusion, respectively. The impact of psychological stress and esophageal acid infusion on masseter muscle and ANS activities was evaluated by determining the changes in masseter muscle and ANS activity between rest (reading a book) and a psychological stress task and between no infusion, esophageal saline infusion, and acid infusion.

METHODS

The study design was approved by the Kagoshima University Hospital and Kagoshima University Ethics Committees (No.22-120, 25-141, and 180009), and all participants provided written informed consent after receiving a full explanation of the study's goals and structure. The study was registered using the University Hospital Medical Network, registry number UMIN000005350 (Object 2: To reveal the effect of stress and esophageal acidification on masticatory muscle activity). In the "*Participants*" subsection of the "*Methods*" section of this paper, there is some overlap with our previous study,⁶ registered as UMIN000005350 (Object 1 in UMIN5350: To reveal the effect of esophageal acidification on masticatory muscle activity).

Participants

Twelve healthy adult men (mean age, 24.1 ± 4.6 years; height, 171.0 ± 7.5 cm; weight, 64.1 ± 6.2 kg) were recruited from the general population through a public advertisement. All participants had complete dentition, without third molars, and had acceptable occlusion. All participants were screened for signs or symptoms of bruxism and temporomandibular disorders assessed by questionnaires and gastrointestinal disorders assessed by esophagogastroduodenoscopy by a gastroenterologist. Individuals with any of the following conditions were excluded: signs or symptoms of bruxism, evidence or history of temporomandibular disorder or gastrointestinal disease as assessed by esophagogastroduodenoscopy and a frequency scale for the symptoms of GERD (FSSG) ≥ 8 points ($n = 3$), clinically significant complaints of heartburn, mental health problems, use of either prescription or over-the-counter drugs within the previous 3 months, and professional education regarding GERD or bruxism.

Experimental Procedure

On the morning after overnight fasting, electromyography (EMG), electrocardiography (ECG), and laryngeal movement assessment were performed using two different tasks: (i) reading a book quietly, as rest, during which the participant was seated in an upright but relaxed position and instructed to read the book;^{6,12} or (ii) calculation, as an ability

task that induces psychological stress, during which participants were given a pre-printed paper and asked to solve calculations involving multiplication and division using pairs of two-digit and three-digit numbers, for 30 min. Three different interventions were performed during those tasks: (i) no infusion, (ii) intra-esophageal saline infusion, and (iii) intra-esophageal acid infusion (0.1 N HCl, pH, 1.2). Thus, six experiments were performed, each lasting 30 min (Fig. 1A). The six-condition experiment was conducted in one day for all participants. All infusions involved administration of 30 mL of fluid at a rate of 1 mL/min,^{6,15,16} and both the acidic and saline solutions were prepared at 37°C to exclude any thermal influence. If acid clearance (intra-esophageal pH > 4) was not confirmed within a 30-min recovery period, the next infusion was not performed until the intra-esophageal pH had reached 4. The order of interventions was determined by a computer-generated randomization list, and the participants were blinded to the order.

To evaluate subjective symptoms, catheter-related nasopharyngeal discomfort and chest discomfort, such as pain or heartburn, were assessed on a 10-point scale (0, no discomfort; 10, intolerable discomfort or pain). Immediately after each intervention, participants were asked to rate their discomfort using a number on the scale.

Data Recording system

All participants underwent polygraphic monitoring consisting of EMG from bilateral masseter muscles and ECG from the CM5 lead under laboratory conditions.⁶ Ag/AgCl disposable electrodes (Blue Sensor N-00-S; METS Co., Tokyo, Japan) were used for all electrophysiological measurements; the electrode-to-skin impedance was lower than 5 k Ω . For further analysis, EMG activity during maximum voluntary tooth clenching (MVC) at the intercuspal position for 3 s and resting for 10 s was measured.¹⁷ Laryngeal movement was measured using a piezoelectric sensor (AP-C029; TEAC, Tokyo, Japan) attached to the skin over the thyroid cartilage.^{6,16,18}

To assess intra-esophageal pH, a dual-antimony pH probe with infusion port (Multi-Use pH-catheter; SYNECTICS MEDICAL, Barcarena, Portugal) was placed transnasally into the esophagus. Two pH sensors (proximal and distal) and an infusion port were placed at 20, 5, and 10 cm above the superior margin of the lower esophageal sphincter (LES), respectively (Fig. 1B).^{6,16} All signals were amplified, filtered, and recorded at a sampling frequency of 1 kHz by means of a polygraph system (PolymateII; TEAC, Tokyo, Japan). Audio–video recordings of the head and upper body were also obtained.

Analysis of Swallowing and Cervicofacial Behavior

Swallowing events were scored according to the signals recorded by laryngeal-movement sensors in addition to EMG activity or visual observation of

laryngeal movement on audio-video data.² Cervicofacial behavior, such as coughing, sniffing, lip sucking, or upper body movement, was identified visually from audio-video data. The frequency of swallowing and cervicofacial behavior (times/min) was calculated.⁶

Analysis of EMG Data

EMG data were full-wave-rectified and normalized by the amplitude during MVC in each participant, and the normalized EMG activity on the right and left sides were averaged. For use as a representative value of the total EMG activity, the EMG data were time integrated (%•s).^{6,16} Behavior-related activity (%•s) was defined as the activity that accompanied swallowing and cervicofacial behavior. Baseline activity (%•s), determined by subtracting the behavior-related activity from the total EMG activity (Fig. 1C), was defined as the activity during the period when no cervicofacial behavior was performed. The duration of baseline (s) and mean baseline activity (%) were also calculated.

Analysis of ECG Data

Heart rate and heart rate variability (HRV) values were obtained from the ECG data using biomedical signal analysis software (Map1060; Nihonsanteku, Osaka, Japan).⁶ Power spectral analysis of HRV was performed by means of fast-Fourier transform. Of

the two main oscillations in HRV, the curve of the spectra within the ranges of 0.04–0.15 Hz and 0.15–0.40 Hz were defined as low frequency power (LFP; ms^2) and high-frequency power (HFP; ms^2), respectively. The low-/high-frequency power ratio (LFP/HFP) and normalized HFP [$\text{HFP} / (\text{LFP} + \text{HFP})$] were used as indices of sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) activities, respectively.^{6,19}

Statistical Analyses

Variables were compared to examine the effect of the tasks and interventions. Significant differences were evaluated using the paired *t*-test or Wilcoxon's signed-rank test, according to the data distribution. A two-way repeated-measures ANOVA was used to examine the main and interaction effects on the outcome measures. Measurements in the different tasks (reading or calculation) and the different interventions (saline infusion or acid infusion) were compared using generalized linear models. Statistical significance was set at $P < 0.05$ after Bonferroni adjustment for multiple comparisons. All statistical analyses were performed using SPSS version 24.0 for Windows (IBM Corporation, Armonk, NY), with significance set at $P < 0.05$. The sample size was smaller; therefore, we performed post-hoc power calculations ($1-\beta$), and we defined *P* values as < 0.05 with a power calculation of > 0.8 as significant.

RESULTS

No significant difference was observed in any variables between the no-infusion and saline-infusion conditions for each task, except for the frequency of swallowing under calculation task (Table 1).

During the rest task (reading a book), baseline and mean baseline activities (masseter muscle activity excluding behavior-related activity) were significantly higher ($P = 0.019$ and 0.032 , respectively; Table 2) and PNS activity ($HFP/[LFP+HFP]$) was significantly lower ($P = 0.021$; Table 2) during acid infusion than during saline infusion. However, during the calculation task, although the frequency of swallowing and frequency of cervicofacial behaviors during acid infusion were higher than those during saline infusion ($P = 0.016$ and 0.032 , respectively; Table 2), there was no significant difference in masseter muscle activity and ANS activity between the saline and acid infusion conditions. During saline infusion, baseline and mean baseline activities and SNS activity (LFP/HFP) under the calculation task were higher than those during the reading task ($P = 0.022$, 0.041 and 0.012 , respectively; Table 2). In contrast, PNS activity was lower during the calculation task than during the reading task ($P = 0.007$; Table 2).

In two-way repeated-measures ANOVA, intra-esophageal infusion (saline or acid) significantly affected baseline and mean baseline activities, frequency of swallowing,

cervicofacial behaviors, and PNS activity ($P = 0.008, 0.012, 0.028, 0.026$ and 0.021 , respectively; Fig2A). However, performing tasks (reading or calculation) significantly affected only PNS activity ($P = 0.028$; Fig2A). No significant interaction effect was found between intra-esophageal infusions and tasks (Fig. 2A, B).

Next, the data analyzed every 5 min were compared for the reading and calculation tasks during saline infusion and for saline infusion and acid infusion during the reading task, respectively. During saline infusion, the baseline activity of the calculation task was increased compared to that during the reading task, within the first 5 min ($P < 0.01$, Fig. 3Aa). Changes in ANS activity occurred after the increase in baseline activity ($P < 0.05$ or 0.01 ; Fig. 3Ba and Ca). However, during reading, the baseline activity during intra-esophageal acid infusion increased during the first 15–20 and 20–25 min compared with that during saline infusion ($P < 0.05$ and 0.01 , respectively; Fig. 3Ab). During intra-esophageal acid infusion, SNS activity increased during the first 10–15 and 15–20 min ($P < 0.05$ and 0.01 ; Fig. 3Bb), and PNS activity decreased during the first 15–20 min ($P < 0.05$; Fig. 3Cb), as compared with the saline infusion. The timing of changes in ANS activity occurred before/just the baseline activity increase (Fig. 3Ab, Bb, and Cb).

DISCUSSION

The results of this study showed that intra-esophageal acid infusion significantly increased masseter muscle activity and decreased PNS activity. In contrast, psychological stress task significantly decreased PNS activity, but only modestly increased masseter muscle activity and SNS activity (Table 2, Fig.2A).

In the present study, we selected healthy adult men as subjects. Autonomic effects of acid infusion have been reported to differ between healthy subjects and GERD patients.²⁰ Additionally, sex modifies the association between GERD and bruxism, with women having a higher risk for GERD-associated bruxism than men.^{21,22} Such sex-specific effects are not unknown; for instance, women have a higher risk for depression and anxiety than men,²³ because of sex differences in genes, hormones, and physiological stress responses.^{24,25} Thus, we selected healthy men who had an FSSG score of less than 8 and who had no GERD symptoms to evaluate the effect of acid infusion and stress without these confounding effects. When examining masseter muscle activity during wakefulness, the task performed by the subject is very important. Acute changes in the myoelectric activity of the masseter and temporalis muscle have been noted under stressful conditions, such as when performing mental arithmetic tasks or other stress-reactivity tasks.²⁶ In another report, the frequency and duration of clenching episodes was higher during a practical ability task (i.e., playing a videogame) than when reading a book.¹²

In this study, during the rest task (reading a book), masseter muscle activity without the behavior-related activity increased and PNS activity decreased during acid infusion as compared to saline infusion. Additionally, during saline infusion, masseter muscle activity without the behavior-related activity and SNS activity under the calculation task increased relative to the reading task. In contrast, PNS activity decreased during the calculation task as compared to during the reading task (Table 2). These results were similar to those of previous reports.^{6,12} Recently, the definition of AB has highlighted two important aspects. First, the focus has shifted to muscle activity, indicating that bruxism does not necessarily involve tooth contact. Second, a recent review suggested that, in otherwise healthy individuals, bruxism should not be considered as a disorder, but rather as a behavior that can be a risk (and/or protective) factor for certain clinical consequences.²⁷ Our study subjects were healthy, without bruxism symptoms. The relationship of masseter muscle activity, caused by a psychological stress task and intra-esophageal acid stimulation, with AB remains unclear. However, GER and a stress task while awake may increase masseter muscle activity; thus, GER and stress should be considered as risk factors for AB.

As two-way analysis in the intervention data during 30-min performing tasks significantly affected only PNS activity. Although performing tasks tended to affect masseter muscle activity and SNS activity ($P = 0.090$ and $P = 0.053$, respectively), the effects were not significant. However, intra-esophageal infusions (saline or acid

infusion) significantly affected masseter muscle activity and PNS activity. Although intra-esophageal infusions tended to affect SNS activity ($P = 0.057$), the effect was not significant. Since no significant interaction effect between intra-esophageal infusions and tasks was found, masseter muscle activity and PNS activity changed due to the intra-esophageal acid infusion, regardless of the task. These results suggest that intra-esophageal acid infusion was a significant factor on masseter muscle activity and PNS activity. Therefore, people with GER are more likely to have increased masseter muscle activity during the day and may be prone to wakeful bruxism.

When we analyzed the intervention data every 5 min, we found differences in the timing of changes in SNS activity and the increase in masseter muscle activity between intra-esophageal acid stimulation and calculation-induced stress. In a previous study with intra-esophageal infusion of 0.15 mol/L hydrochloric acid or saline at a constant rate of 8 mL/min for 30 min, the SNS activity averaged over the first 5 min of infusion was already greater under acid infusion than that under saline infusion.²⁸ PNS activity averaged over the last 5 min of infusion was lower under acid infusion than under saline infusion. Additionally, acid-induced esophageal hyperalgesia correlated with reduced PNS activity. In the present study, the SNS activity increase and PNS activity decrease were seen after 10–20 min under acid infusion. The difference between these study results may be related to the smaller amount of infused solution in the present study than in the previous study, and the lack of pain experienced by the

subjects under the acid infusion. We consider that those ANS activity changes under intra-esophageal acid stimulation were caused by the chemical (acidic) stimulation, as saline infusion did not induce those changes and chest discomfort, including pain or heartburn, was not increased by acid infusion. Refluxed gastric acid can penetrate within the intercellular junctional complexes in the surface layers of the esophageal (stratified squamous) epithelium, where it encounters and activates chemosensitive nociceptors whose signals are transmitted via the spinal cord to the brain, leading to perception of symptoms (heartburn).²⁹ The small amounts of acid solution used in this study gradually penetrated between the cells of the surface layers of the esophageal epithelium during infusion, which may have caused changes in ANS activity.

During intra-esophageal acid infusion, masseter muscle activity increased immediately after ANS activity change under intra-esophageal acid stimulation. It has been reported that masseter muscle activity is closely related to changes in ANS activity. Sympathetic nerves derived from the superior cervical sympathetic trunk (CST) that supplies blood vessels in the orofacial area have been reported to induce vasoconstrictor responses in the masseter muscles of a number of animal species.³⁰⁻³³ Additionally, sympathetic nerves from the CST have been reported to interact with parasympathetic nerves in the regulation of blood flow to orofacial tissues in cats^{34,35} and dog.³⁶ Thus, our results suggest that changes in ANS activity induced by intra-esophageal acid stimulation may cause an increase in masseter muscle activity.

The increase in masseter muscle due to calculation, however, was more rapid than the changes in ANS activity due to calculation. Psychological stress is mediated by two main pathways: the hypothalamic–pituitary–adrenal axis (neuroendocrine route) and ANS (neural route).³⁷ It is difficult to clarify how the endocrine pathway is related to the EMG activity of the masseter muscle as it relates to psychological stress. SNS may modulate muscle spindle afferent activity, mostly in a tonic manner, and the SNS could participate reflexively in modifying muscle tone.³⁸ Sympathetic activation would exert a powerful depressant action on jaw jerk and tonic vibration reflexes.³⁸ This indicates a reduction in spindle sensitivity to changes in muscle length.³⁹ Thus, the SNS has both active and negative effects on the masseter muscle activity.

In the present study, ANS activity changes due to the psychological stress task averaged over the first 10 min only may have been unmeasurable owing to the complexity of the SNS. However, another previous study has reported that stress during a 15-s mental arithmetic trial increases masseter muscles activity.¹³ It was unclear whether such fast reaction was noted in our study results. However, an increase in masseter muscle activity occurred at the first 5 min after the start of the psychological stress task; thereafter, changes in ANS activity were observed,

suggesting that the psychological stress task may cause rapid masseter muscle activity increase, whereas the changes in ANS may not be rapid.

It has been reported that forward head posture increased the masseter muscle activity.⁴⁰ However, in this study, the position of the head was not fixed because it was thought that this could contribute as a stress factor.¹³ We confirmed the head posture 5 min after beginning each task with no infusion. Consequently, the mean \pm SD inclination of the Frankfurt plane relative to the floor during stress task or reading task was $24.5 \pm 9.6^\circ$ or $21.3 \pm 10.9^\circ$, respectively. There was no significant difference between the head posture during stress task and that during reading task, according to paired *t*-test. Additionally, since arm and hand movements such as turning the pages of a book and writing answer on the paper occurred during each task, in our pilot study, we confirmed that the changes induced in masseter muscle activity and ANS activity while performing different arm and hand movements (turning the pages of a book without reading and writing number on the paper without calculation) were slight. Thus, we think that different head postures and arm and hand movements while performing calculation and reading tasks did not affect the masseter muscle activity and ANS activity in this study.

Our study had some limitations. First, the sample size was very small. We defined the significance set at P values as < 0.05 with a power calculation $(1-\beta)$ of > 0.8 as significant to avoid Type II errors (false negative results). However, Type I errors (false positive results) could not be avoided. Thus, if the sample size is large, there may be detectable results. Second, it was unclear whether calculation task induced psychological stress and/or whether reading task did not induce stress, although the calculation task increased SNS activity and decreased PNS activity compared with the reading task. Moreover, we did not consider chronic stress, i.e. work or social overloads and pressure to perform, of the subjects before this study. Further studies in subjects with similar levels of chronic stress and objective assessment of the degree of stress is needed to clarify the type of stress associated with masseter muscle activity and ANS activity. Finally, we could not determine the causal relationships of intra-esophageal acid stimulation and psychological stress with ANS activity change and masseter muscle activity increase. To elucidate the mechanism behind the masseter muscle activity increase, an animal study is required.

In summary, the results of this study showed that intra-esophageal acid stimulation and psychological stress in the form of a calculation task increased masseter muscle activity, which may be related to ANS activity. Whereas esophageal acid infusion had

a significant effect on masseter muscle activity and PNS activity, stress task only had a significant effect on PNS activity. Thus, in patients with awake bruxism, examining the symptoms of GER might help to identify and treat the causative agent of awake bruxism.

CONCLUSION

Our results suggested that intra-esophageal acid infusion significantly increased masseter muscle activity and decreased PNS activity. In contrast, psychological stress task only significantly decreased PNS activity. Whereas psychological stress task increased masseter muscle activity and SNS activity, these effects were not statistically significant. Additionally, since the timing of the increase in masseter muscle activity and change in ANS activity differed between intra-esophageal acid stimulation and the stress task, the pathway for increased masseter muscle activity may differ between these two conditions.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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Figure Legends

Figure 1. Experimental design and data analyses (according to Ohmure et al. 2014).

(A) Timeline of the experiment. The triangular marks indicate the timing of subjective symptom assessment. (B) Schema of the positioning of pH sensors and the infusion port. (C) Examples (reading task during no infusion, reading task during acid infusion, and calculation task during no infusion) of esophageal pH, masseter muscle activity, and laryngeal movement in a study participant. The black and white boxes indicate the segments that were regarded as behavior-related and baseline activities (masseter muscle activity excluding behavior-related activity), respectively.

BM, laryngeal movement associated with upper body movement; LES, lower esophageal sphincter; MVC, maximum voluntary tooth clenching; SNF, laryngeal movement associated with sniffing; SWL, laryngeal movement induced by swallowing

Figure 2. Main effect and interaction effect analysis of task and intra-esophageal infusion for each measurement. (A) Results of the two-way repeated-measures ANOVA with different tasks (reading or calculation) and different intra-esophageal infusions (saline or acid) as factors. (B) Results showing lack of interaction effect between task (reading or calculation task) and intra-esophageal infusion (saline or

acid) on (a) baseline activity (masseter muscle activity excluding behavior-related activity) and (b, c) ANS activity (b: SNS activity (LFP/HFP); c: PNS activity (HFP/(LFP+HFP))).

ANS, autonomic nervous system; HFP, high-frequency power; LFP, low-frequency power; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

Figure 3. Analysis of the changes recorded every 5 min under the calculation task and acid infusion. **(A)** Baseline activity (masseter muscle activity excluding behavior-related activity) and **(B, C)** ANS activity (B: SNS activity (LFP/HFP); C: PNS activity (HFP/(LFP+HFP))) on (a) the reading task or calculation task during saline infusion and (b) saline infusion or acid infusion during the reading task.

ANS, autonomic nervous system; HFP, high-frequency power; LFP, low-frequency power; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

Significance was determined by the paired *t*-test or Wilcoxon's signed-rank test (**P* < 0.05, ***P* < 0.01).

Table 1. Comparison of masseter muscle activity, swallowing, cervicofacial behavior, and ANS activity between no infusion and intra-esophageal saline infusion during the reading or calculation task (n = 12).

Variable	Reading task			Calculation task		
	No infusion	Saline infusion	P^{\dagger}	No infusion	Saline infusion	P^{\dagger}
	Mean \pm SD	Mean \pm SD		Mean \pm SD	Mean \pm SD	
Masseter muscle activity						
Total activity (% \cdot s)	4105 \pm 2852	4187 \pm 2601	0.583	4887 \pm 2407	5033 \pm 1784	0.875
Behavior-related activity (% \cdot s)	1086 \pm 1828	1203 \pm 1368	0.347	963 \pm 901	1023 \pm 758	0.814
Baseline activity (% \cdot s)	3019 \pm 1488	2984 \pm 1513	0.792	3924 \pm 1694	4010 \pm 1404	0.804
Mean baseline activity (%)	1.8 \pm 0.9	1.8 \pm 0.9	0.787	2.3 \pm 1.0	2.3 \pm 0.8	1.000
Cervicofacial behaviour						
Frequency of swallowing (times / min)	0.9 \pm 0.4	0.9 \pm 0.4	0.877	0.8 \pm 0.1	0.9 \pm 0.2	0.024
Frequency of cervicofacial behaviours (times / min)	0.2 \pm 0.3	0.2 \pm 0.2	0.504	0.2 \pm 0.2	0.3 \pm 0.4	0.323
ANS activity						
Sympathetic: LFP/HFP	2.27 \pm 1.44	2.02 \pm 1.55	0.433	2.47 \pm 1.33	2.75 \pm 1.64	0.103
Parasympathetic: HFP/(LFP+HFP)	0.36 \pm 0.14	0.40 \pm 0.19	0.096	0.33 \pm 0.14	0.32 \pm 0.15	0.277
Subjective psychological symptoms						
Chest discomfort, including pain or heartburn	2.58 \pm 3.12	3.08 \pm 3.12	0.317	1.75 \pm 2.44	1.50 \pm 1.93	0.458

ANS, autonomic nervous system; HFP, high-frequency power; LFP, low-frequency power.

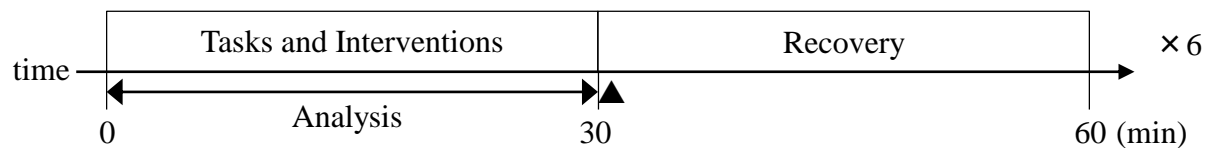
[†] Significance was determined by the paired *t*-test or Wilcoxon’s signed-rank test.

Table 2. Comparison of masseter muscle activity, swallowing, cervicofacial behavior, and ANS activity between intra-esophageal infusion of saline and acid during the reading or calculation task (n = 12).

Variable	Reading task			Calculation task			Reading task vs. Calculation task	
	Saline infusion	Acid infusion	<i>P</i> [†]	Saline infusion	Acid infusion	<i>P</i> [†]	Saline infusion	Acid infusion
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		<i>P</i> [†]	<i>P</i> [†]
Masseter muscle activity								
Total activity (% · s)	4187 ± 2601	5180 ± 3558	0.104	5033 ± 1784	6053 ± 2744	0.182	0.158	0.407
Behavior-related activity (% · s)	1203 ± 1368	1218 ± 1602	0.814	1023 ± 758	1266 ± 1216	0.433	0.695	0.937
Baseline activity (% · s)	2984 ± 1513	3962 ± 2135	0.019	4010 ± 1404	4787 ± 1800	0.064	0.022	0.253
Mean baseline activity (%)	1.8 ± 0.9	2.3 ± 1.3	0.032	2.3 ± 0.8	2.8 ± 1.1	0.099	0.041	0.266
Cervicofacial behaviour								
Frequency of swallowing (times / min)	0.9 ± 0.4	1.3 ± 0.7	0.065	0.9 ± 0.2	1.2 ± 0.3	0.016	0.878	0.516
Frequency of cervicofacial behaviours (times / min)	0.2 ± 0.2	0.1 ± 0.1	0.255	0.3 ± 0.4	0.2 ± 0.3	0.032	0.717	0.635
ANS activity								
Sympathetic: LFP/HFP	2.02 ± 1.55	2.62 ± 1.44	0.136	2.75 ± 1.64	2.87 ± 1.58	0.458	0.012	0.460
Parasympathetic: HFP/(LFP+HFP)	0.40 ± 0.19	0.32 ± 0.14	0.021	0.32 ± 0.15	0.30 ± 0.14	0.306	0.007	0.446
Subjective psychological symptoms								
Chest discomfort, including pain or heartburn	3.08 ± 3.12	3.67 ± 2.96	0.317	1.50 ± 1.93	2.38 ± 2.13	0.102	0.197	0.268

ANS, autonomic nervous system; HFP, high-frequency power; LFP, low-frequency power.

[†] Significance was determined by the paired *t*-test or Wilcoxon's signed-rank test.

A

Task 1: Reading task (as rest)

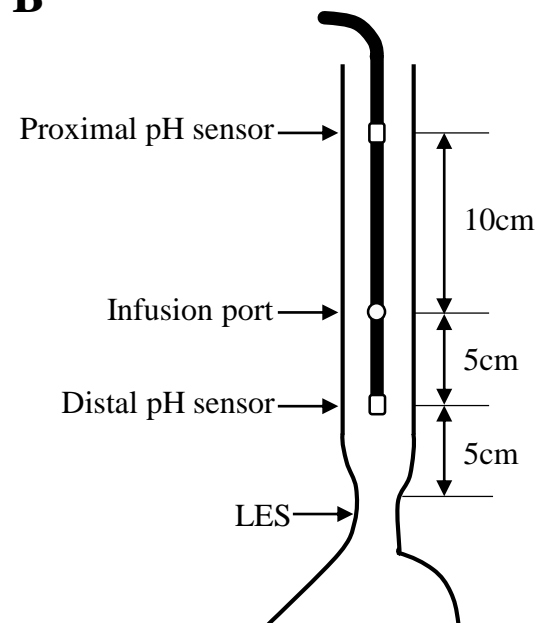
Task 2: Calculation task (as an ability task-induced psychological stress)

Intervention 1: No infusion

Intervention 2: Intra-esophageal saline infusion

Intervention 3: Intra-esophageal acid infusion (0.1 N HCl; pH 1.2)

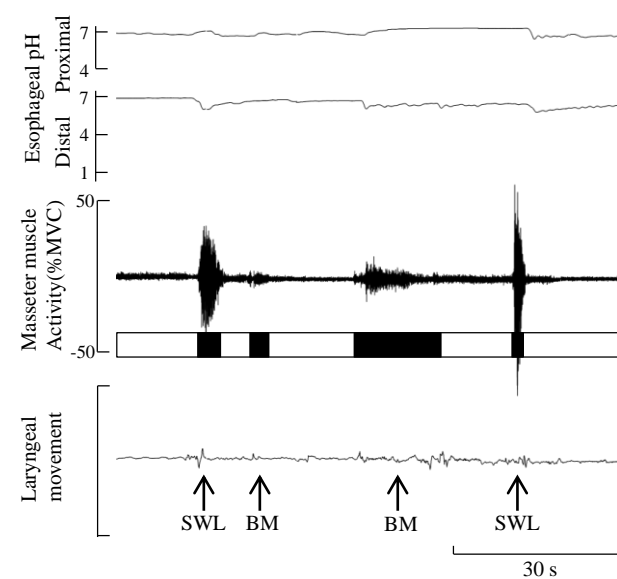
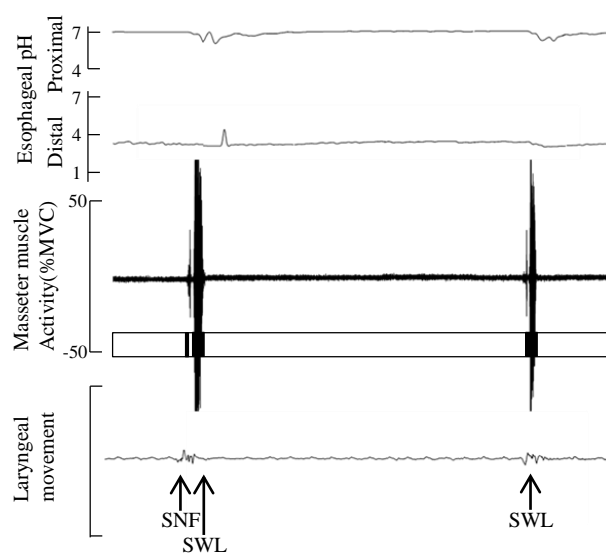
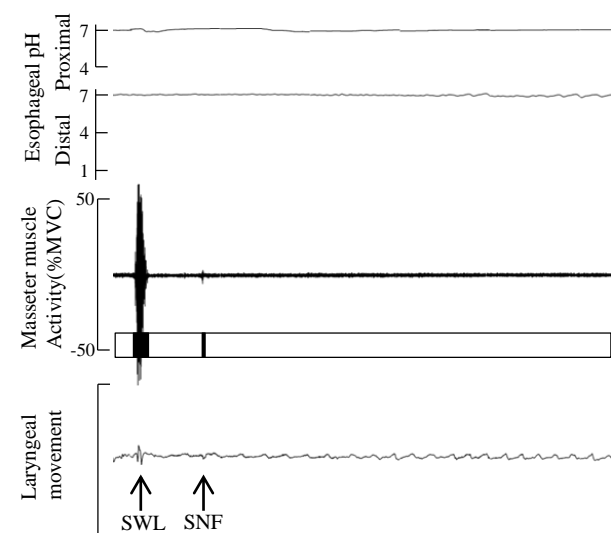
▲: The timing of subjective symptom assessment

B**C**

Reading task during no infusion

Reading task during acid infusion

Calculation task during no infusion



A

Results of the two-way repeated-measures ANOVA with different tasks (reading or calculation) and different intra-esophageal infusions (saline or acid) as factors.

Dependent variables	Main effect				Interaction	
	Different tasks (Reading or Calculation)		Different intra-esophageal infusions (Saline or Acid)		Different tasks (Reading or Calculation) × Different intra-esophageal infusions (Saline or Acid)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Masseter muscle activity						
Total activity (% • s)	1.372	0.266	4.501	0.057	0.001	0.970
Behavior-related activity (% • s)	0.045	0.835	0.307	0.591	0.675	0.429
Baseline activity (% • s)	3.445	0.090	10.442	0.008	0.169	0.689
Mean baseline activity (%)	3.102	0.106	8.936	0.012	0.070	0.797
Cervicofacial behavior						
Frequency of swallowing (times / min)	0.193	0.669	6.420	0.028	0.698	0.421
Frequency of cervicofacial behaviors (times / min)	0.324	0.581	6.569	0.026	0.006	0.938
ANS activity						
Sympathetic: LFP/HFP	4.691	0.053	4.540	0.057	2.347	0.154
Parasympathetic: HFP/(LFP+HFP)	6.410	0.028	7.301	0.021	4.826	0.050

ANS, autonomic nervous system; HFP, high-frequency power; LFP, low-frequency power.

