Introduction
Following the Great East Japan Earthquake in 2011, many rescue workers suffered from post-traumatic stress disorder (PTSD) caused by emotional stress\(^3\). Ameliorating the stress level of rescue workers during their duties would significantly reduce the number who suffer from PTSD. Among possible stress stimuli in a disaster situation, somatosensory and olfactory stressful stimuli can be dampened by wearing protective clothing such as thick gloves and protective masks\(^2\). However,
there are no means for avoiding or dampening visually-evoked stress, because visual information is essential for search and rescue operations in a disaster situation.

Previous animal and human research suggest a possible role of active mastication in reducing stress responses. Tahara et al. demonstrated that light clenching reduces salivary cortisol levels during a mental arithmetic task. Their study suggests that clenching could be an applicable stress-relieving approach for rescue workers without interfering with their work; however, the effect of clenching on visually-evoked emotional stress responses has yet to be determined. We therefore investigated whether active clenching during exposure to visual stress affects systemic stress responses in young adults. We continuously monitored electrocardiograms (ECG) to investigate visual stress responses in an experimental disaster environment in which provocative and unpleasant pictures that contained mutilated or injured bodies were presented. We determined the time-course changes in the heart rate (HR) to study whether clenching during exposure to stressful pictures can modulate fear bradycardia, an instantaneous decrease of heart rate known as a physiological index of freezing-like defensive behavior. Fear bradycardia is a parasympathetically-dominated autonomic defensive response through a neural projection from the amygdala to the periaqueductal gray (PAG), and is implicated in the development and maintenance of psychopathology in humans. Using heart rate variability analysis, we also determined the cardiac sympathetic and parasympathetic responses to the visually-evoked stresses, and investigated the causal relationship of how clenching affects autonomic nervous responses to suppress fear bradycardia.

Materials and Methods

1. Participants
Twenty-five young male adults (22.2 ± 0.3 years old) participated in the experiment. The study followed the protocol for the use of human participants and was approved by the Ethics Committee of Kanagawa Dental University (Approval no. 334).

2. Visual stimuli
Stimuli comprised 20 color pictures that were selected from the International Affective Picture System (IAPS). Ten negative pictures contained provocative and unpleasant images of mutilated or injured bodies to simulate visually-evoked emotional stress in a disaster situation, while the other neutral pictures contained normal bodies or body parts. All pictures were randomly presented on a 12.1-inch LCD monitor that was placed in front of the participant, each for 5 s with 25 s of inter-stimulus interval between stimuli. A white fixation cross on a black background was presented at the center of the screen during the interval. Participants evaluated the emotional valence and arousal levels of each picture using the nine-grade SAM system (the Self-Assessment Manikin, 9: pleasant, 1: unpleasant for emotional valence and 9: high arousal, 1: no arousal for arousal) at the completion of the ECG recording. The emotional valence was used to evaluate the strength of the pleasant/unpleasant feeling that was accompanied by perceivable physiological responses such as palpitation.

3. Clenching intervention
The participants watched 20 pictures under ECG measurement two times on different experimental days, which were at least two weeks apart from each other. We used N2101, N2107, N2442, N2445, N2525, N7507, N7509, N7512, N7513, and N9260 as negative pictures and S3000, S3010, S3051, S3053, S3060, S3069, S3110, S3150, S3250, and S3400 as neutral pictures. On either experimental day, a custom-made, 1-mm-thick soft mouthpiece (Fig. 1) was attached to the maxilla of the participant and they were instructed to gently clench the mouthpiece during picture presentation regardless of the content of the picture (with-clench
condition). Electromyograms (EMG) from the right masseter were recorded concurrently with ECG using the telemetry system (WEB-1000; Nihon Koden Ltd., Tokyo, Japan) to monitor clenching activity, and the participants were instructed to clench within 10–20% of their maximum strength with visual feedback of EMG activity before the picture viewing session. The participants also performed an extra 'clenching-only' session after the picture viewing session, which consisted of five clenches according to the block design as described above but without visual stimuli (sole-clench condition). A fixation cross was presented for the whole session and the experimenter noted the beginning and the end timing of clenching by gently touching the participant’s upper right arm. On the other experimental day, the mouthpiece was not attached and the participants were instructed not to clench during the experiment (without-clench condition). The order of the two experimental conditions was randomized and counter-balanced among participants.

4. ECG recording and HRV analysis

ECG in the lead II configuration was measured at a sampling rate of 1 kHz with a 1.6–30 Hz band-pass filter throughout the experiment using the same telemetry system. We investigated whether masticatory intervention altered HR, sympathetic and parasympathetic activity under visually-evoked stress. HRV was evaluated in the frequency domain\(^{10}\), which has been widely accepted as a reliable noninvasive method for assessing cardiac autonomic control\(^{11,12}\). We used in-house developed software running on Matlab (Mathworks, Natick, MA) to measure HR (beats/min) and R-R intervals on a beat-to-beat basis\(^{13}\). Briefly, the detected R-R interval data were first resampled at 60 Hz (Fig. 2a). We excluded the periods in which we could not detect the time points of R waves because of motion artifacts. Continuous wavelet transform analysis based on Morlet wavelets was then performed to determine the time-course of changes in the autonomic activity. We determined low-frequency activity (LF) as the integrated total of spectral power between 0.04–0.15 Hz and high-frequency activity (HF) as that between 0.15–0.4 Hz (Fig. 2b). We used the HF and the ratio LF/HF as markers of parasympathetic and sympathetic activity, respectively\(^{11,12}\). The sympathetic and parasympathetic responses were further averaged over the trials for statistical comparison.

5. Statistics

Emotional valence and arousal levels were compared between contexts (neutral and negative) or conditions (with and without clenching) using the Wilcoxon signed rank test because of their deviation from normality confirmed by the Lilliefors normality test. HR, sympathetic activity, and parasympathetic activity were first normalized based on the values in the 5 s before picture presentation and are shown as a ratio to the baseline (resting) state. Either the paired t-test or the Wilcoxon signed rank test was chosen, depending on the results of the Lilliefors normality test, to compare the statistical difference of the normalized values between with- and without-clench conditions or picture context at each time point. To further investigate the interaction between cardiac responses evoked by emotional pictures and that by clenching, pseudo-clench condition
Results

1. Subjective valence and arousal levels for negative and neutral visual stimuli

The mean emotional valences of neutral and negative pictures were 5.53 ± 0.13 and 2.53 ± 0.13 in the without-clenching condition and 5.62 ± 0.18 and 2.35 ± 0.17 in the with-clenching condition, respectively. The mean arousal levels for neutral and negative pictures were 2.46 ± 0.30 and 5.70 ± 0.33 in the without-clenching condition and 2.26 ± 0.26 and 6.21 ± 0.30 in the with-clenching condition, respectively. There was a statistically significant decrease in emotional valence and increase in arousal while viewing negative pictures compared with neutral pictures (p < 0.01) in both conditions. These results indicate that negative pictures evoked strong unpleasantness and arousal compared with neutral pictures, as reported previously, regardless of the clenching intervention. Although there was a tendency for clenching to increase arousal while viewing negative pictures (p = 0.055; with v.s. without clenching conditions in negative picture viewing), clenching did not affect the subjective sense of emotional valence or arousal.

2. Visually-evoked stress-induced fear bradycardia

Figure 3 illustrates the mean HR, LF/HF, and HF of HRV during neutral or negative picture viewing. We found a decrease in HR only during negative picture viewing, indicating a fear bradycardia response (Fig. 3a and d, without-clench). Because HF responses kept increasing in the same time period (Fig. 3f), this bradycardia response was likely to be brought on by an increased parasympathetic tone during stressful visual stimuli, as reported previously. The LF/HF response was almost stable during the picture viewing period and the following rest period in neutral picture viewing (Fig. 3b); however, in the case of negative picture viewing it gradually increased from the middle of the viewing period and reached its peak at around 5 s after the end of picture viewing (Fig. 3e). Because the time-course of the LF/HF peak corresponds to the rebound of HR after picture viewing (Fig. 3d), the increase in LF/HF response may be the compensatory activity of the sympathetic nervous system triggered by the preceding enhancement of parasympathetic tone that induced fear bradycardia.
3. Clenching suppressed fear bradycardia induced by negative picture stimuli

Clenching during picture viewing increased HR regardless of the context of the picture (Fig. 3a and d). We found a statistically significant difference in HR between with- and without-clenching conditions 2.7–5.3 s and 2.8–5.9 s from the beginning of neutral and negative picture presentation, respectively. It is notable that clenching counteracted the decrease in HR in the negative visual stimuli (Fig. 3d). Although no comparison of HRV indices between conditions reached statistical significance, we found a tendency for clenching to suppress the increase of HF responses during negative picture presentation (2.5–5.2 s from the beginning of neutral and negative picture presentation, \( p < 0.08 \); Fig. 3f). These results suggest that clenching mitigated the rapid increase of parasympathetic responses to the negative visual stimuli and prevented fear bradycardia.

4. Clenching may interact with the autonomic nervous response to negative visual stimuli and prevent fear bradycardia

The significant effect of clenching on fear bradycardia motivated us to investigate the hypothesis that autonomic responses to visual stimuli and clenching actively interact with each other to mitigate fear bradycardia. Figure 4 shows HR and HRV indices comparing the actual and pseudo-clench conditions. Although the outcome HR responses were almost the same (Fig. 4a and d), we found significant differences in the responses of the LF/HF and HF components in both the neutral and negative picture stimuli, especially during the post-stimulus period (later than 10 s from the end of picture viewing in Fig. 4b, c, e and f). A significant difference in HF responses between actual and pseudo-clench conditions during picture presentation was found only for negative picture viewing (Fig. 4f). These results indicate that the effect of clenching on fear bradycardia is not a mere summation of independent autonomic responses to visual stress and those to clenching, suggesting the interaction of autonomic activity driven by clenching and visual stimuli.

**Discussion**

We investigated how masticatory stimuli during visual stress exposure affect the systemic stress response of fear bradycardia. Clenching during stressful visual stimuli prevented an acute decrease in heart rate, possibly via dampening the parasympathetic response to the fearful stimuli. Allen and Lauterbach\(^{14} \) reported that cumula-
tive exposure to trauma modulates autonomic defense behaviors to develop an enhanced freezing response to fearful stimuli in addition to overall hypervigilance\(^{15}\), which is in line with PTSD phenomenology\(^{16}\). These papers suggested that a repetitive experience of fear bradycardia may cause plastic changes in the systemic emotional responses to stressful events, triggering the development of psychopathology. Preventing fear bradycardia by active clenching could be useful to protect rescue workers from the cumulative experience of fear bradycardia and help them to avoid developing stress-related diseases.

Our previous animal studies demonstrated that masticatory stimuli under emotional stress inhibited the activation of the hypothalamic-pituitary-adrenal axis to reduce the endocrine stress response\(^{17}\) as well as the autonomic stress responses\(^{18}\). The stress-relieving effect of masticatory stimuli was further preserved in the current human study of visually-evoked emotional stress. Together with the previously reported inhibitory effect on salivary cortisol levels during mental arithmetic tasks\(^4\), masticatory stimuli may play a significant role in ameliorating systemic stress responses in a bottom-up manner. Masticatory activity may work as a natural defensive behavior because increased biting force has been reported in human participants under emotional stress\(^{19}\).

Masticatory stimulation enhanced HR during picture viewing regardless of the context of visual stimuli (Fig. 3a and d), however the modulatory effect on sympathetic and parasympathetic activity seemed to be different depending on the context. Sympathetic responses (LF/HF) demonstrated a context-non-specific increase, which is consistent with previous research indicating masticatory enhancement of sympathetic nervous activity in a simple gum-chewing task without any cognitive load\(^{20}\). In contrast, the parasympathetic response (HF) was suppressed only while viewing negative pictures (Fig. 3f), which was not explained by the linear summation of time-course responses of separate conditions (pseudo-clench; Fig. 4f). The hypoactivation of parasympathetic responses under emotional stress is a novel and important finding and allows us to understand the mechanism of how mastication modulates stress responses, because most previous human and animal studies have highlighted the masticatory effect on stress perception as suppressing the hyperactivation of sympathetic nervous activity\(^2\)\(^{18}\). The difference between previous stress protocols and the current one was the nature of the stress context; the former induces the ‘fight or flight’ response (tachycardia) while the latter induces the freezing response (bradycardia). Our current results suggest that masticatory stimulation acts to maintain homeostatic control of the autonomic balance by modulating either sympathetic or parasympathetic nervous activity.

The subjective ratings of emotional valence and arousal levels were comparable regardless of the clenching intervention despite the significant effect on fear bradycardia. The discrepancy between physiological responses and subjective ratings has been reported previously in experimental settings using similar IAPS visual stimuli\(^{15}\). The extremely unpleasant nature of negative pictures might override the effect of clenching on mood, considering the positive effect of mastication on state-trait anxiety\(^{21}\). Other physiological indices such as salivary cortisol would further confirm the stress-relieving effect of clenching against visual stress.

A limitation of the current study is that we employed only highly unpleasant and unprovocative neutral picture categories without a gradation of emotional valence and arousal levels, which might cause an expectation and/or attentive response. However, bradycardia was only observed in the case of negative picture viewing, indicating that the change in HR is not an orienting effect. Another interesting question is how the masticatory effect in the maintenance of homeostatic control affects systemic responses to other emotional stimuli, such as pleasant pictures.

**Conclusions**

Clenching during a simulated disaster situation prevents fear bradycardia, suggesting the possible function of mastication as an easy and cost-effective mental protection strategy for rescue workers. Further research combining HR measurement and neuroimaging would reveal the causal relationship between how masticatory stimuli affect the amygdala-PAG circuit of fear responses to dampen fear bradycardia.

**Conflict of interest statement**

The authors declare that they have no competing interests.

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References


