J Physiol Sci (2018) 68:175–190
https://doi.org/10.1007/s12576-017-0522-3

ORIGINAL PAPER

Facial skin blood flow responses during exposures to emotionally charged movies

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Received: 20 August 2016 / Accepted: 10 January 2017 / Published online: 21 January 2017

Abstract The changes in regional facial skin blood flow and vascular conductance have been assessed for the first time with noninvasive two-dimensional laser speckle flowmetry during audiovisually elicited emotional challenges for 2 min (comedy, landscape, and horror movie) in 12 subjects. Limb skin blood flow and vascular conductance and systemic cardiovascular variables were simultaneously measured. The extents of pleasantness and consciousness for each emotional stimulus were estimated by the subjective rating from −5 (the most unpleasant; the most unconscious) to +5 (the most pleasant; the most conscious). Facial skin blood flow and vascular conductance, especially in the lips, decreased during viewing of comedy and horror movies, whereas they did not change during viewing of a landscape movie. The decreases in facial skin blood flow and vascular conductance were the greatest with the comedy movie. The changes in lip, cheek, and chin skin blood flow negatively correlated ($P < 0.05$) with the subjective ratings of pleasantness and consciousness. The changes in lip skin vascular conductance negatively correlated ($P < 0.05$) with the subjective rating of pleasantness, while the changes in infraorbital, subnasal, and chin skin vascular conductance negatively correlated ($P < 0.05$) with the subjective rating of consciousness. However, none of the changes in limb skin blood flow and vascular conductance and systemic hemodynamics correlated with the subjective ratings. The mental arithmetic task did not alter facial and limb skin blood flows, although the task influenced systemic cardiovascular variables. These findings suggest that the more emotional status becomes pleasant or conscious, the more neurally mediated vasoconstriction may occur in facial skin blood vessels.

Keywords Emotional challenges · Two-dimensional laser speckle flowmetry · Limb skin blood flow · Emotional and mood status

Introduction

To quantitatively estimate an emotional or mood status in terms of the changes in physiological variables remains to be studied. Fear-induced stress increased heart rate (HR), arterial blood pressure (AP), and limb skeletal muscle blood flow, while limb cutaneous blood flow and surface temperature were decreased [1–6]. Exposure to emotionally charged images, however, did not accompany such systemic cardiovascular responses and limb cutaneous vasoconstriction [7]. Although skin sympathetic nerve activity recorded from the common peroneal nerve increased during the emotional stimulation, the responses in the skin sympathetic nerve activity could not distinguish positively and negatively charged emotional conditions [7, 8]. Thus, systemic cardiovascular variables and limb skin sympathetic nerve activity and blood flow may not be sufficiently sensitive as a physiological estimate for an emotional or mood status.

Facial cutaneous blood vessels are richly innervated by both sympathetic and parasympathetic nerves [9, 10]. The facial parasympathetic nerve may have a vasodilator function, because electrical stimulation of the chorda tympani and lingual nerves caused an increase in feline lip skin blood flow [11] and a vasodilator response in the

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forehead during gustatory intake of chilies was impaired by a lesion of the facial parasympathetic nerve in humans [12, 13]. On the other hand, thermoregulatory intervention revealed that vasomotor function of the facial sympathetic nerve is more complex than that of the parasympathetic nerve. Cutaneous vasodilatation occurred in all facial regions during body heating, while cutaneous vasoconstriction appeared only in the nose region during body cooling [12, 14]. A unilateral lesion in the facial sympathetic pathway diminished the vasodilator response to the thermoregulatory intervention [12]. Thus, the facial sympathetic nerve may have not only vasoconstrictor but also vasodilator functions [9, 12, 14, 15]. A neurally mediated increase in facial skin blood flow may be caused by activation of parasympathetic and sympathetic vasodilator nerves and partly by withdrawal of sympathetic vasoconstrictor activity. On the other hand, a decrease in facial skin blood flow may be due to activation of the sympathetic vasoconstrictor nerve.

The effects of emotional interventions on facial skin blood flow remain little known, although it is empirically known that anger or embarrassment evokes not only facial expression but also flushing. Emotional expression of anger or embarrassment accompanied an increase in forehead skin blood flow, which was diminished by a unilateral lesion in the sympathetic pathway to the face [12, 16, 17]. However, Hayashi et al. [6] reported that fear-induced mental stress by viewing a horror movie did not change forehead skin blood flow and vascular resistance. The discrepant responses in forehead skin blood flow to the negatively charged emotional stimulation may be partly explained by a difference in the type and/or strength of the emotional interventions.

Differently from the variant response in forehead skin blood flow, skin blood flow in the remaining facial regions may exhibit a more consistent response to an emotional intervention, because a substantial difference in the changes of regional facial skin blood flows during nociceptive tooth stimulation was noticed [18]. Therefore, we hypothesized that a negatively charged emotional stimulus may increase facial skin blood flows except for the forehead region, whereas a positively charged emotional stimulus may decrease facial skin blood flow as opposed to negatively charged emotional stimulus. To our knowledge, the effects of emotionally charged stimulation on regional facial blood flows have never been studied. Furthermore, we supposed that facial skin blood flow is a more sensitive physiological variable for reflecting an emotional or mood status in physiological and pathophysiological conditions. A mental arithmetic task, which is used as a stress test, usually influences the cardiovascular variables. Nordin [19] reported using microneurography that an arousal stimulus or mental stress caused an increase in skin sympathetic nerve activity of the supraorbital nerve, which was followed by a cutaneous vasodilator response in the forehead. However, assuming that mental stress may not always cause emotional changes such as a pleasantness-unpleasantness axis, it is expected that skin blood flow in other facial regions may not respond to a mental arithmetic task. The present study aimed (1) to examine noninvasively the effects of audiovisually elicited emotional challenges on regional skin blood flows in the face, using two-dimensional laser speckle flowmetry, (2) to determine which of the changes in facial and limb skin blood flows and systemic cardiovascular variables had the most significant correlation with the subjective ratings of pleasantness and consciousness, and (3) to examine whether mental stress was able to change facial skin blood flows without accompanying emotional changes. A preliminary part of this work has been reported [20].

Methods

Subjects

Twelve healthy volunteers (7 males and 5 females; age, 22 ± 0.3 years; height, 161 ± 3 cm; body weight, 56 ± 2 kg) participated in the present study. None of the subjects suffered from any known cardiovascular and neuromuscular diseases, and they did not take any medications. The experimental procedures and protocols were performed in accordance with the Declaration of Helsinki and approved by the Institutional Ethical Committee of Hiroshima University (permit no. 1425). The subjects gave their informed written consent prior to the experiments. All experiments were performed in a thermoneutral and soundproof environment (room temperature, 25 ± 0.4 °C; relative humidity, 55 ± 2%).

Measurements of facial and limb skin blood flows

Regional facial skin blood flows were monitored in all 12 subjects over the lower part of the face using a two-dimensional laser speckle flowmeter with a line-sensing image device (LFG-1, Softcare Co., Fukuoka, Japan) as shown in Fig. 1. An area of 200 × 200 mm including the face was scanned at 200 × 200 pixels over a time period of 12 s; the spatial resolution was therefore 1 mm. The time difference between the first and last line images was ~12 s. Limb skin blood flow was monitored in 6–7 out of the 12 subjects with a laser Doppler flowmetry instrument (ALF21, ADVANCE Co., Tokyo, Japan), whose probe was placed on the right forearm and on the dorsum of the right hand. The analog voltage signals of the Doppler blood flows were time-averaged with a time constant of 0.1 s.

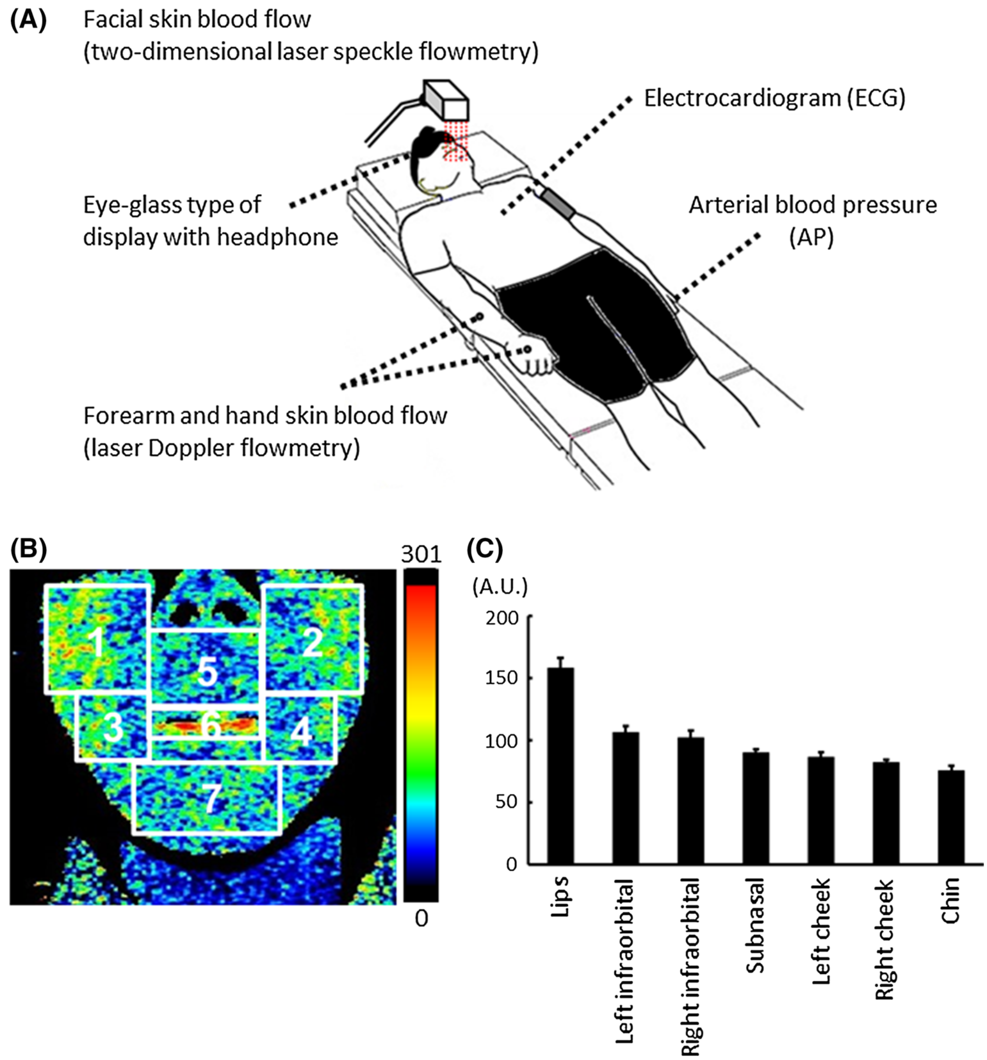


Fig. 1 **a** The experimental setup. **b** An example of two-dimensional laser speckle measurements of skin blood flow in seven different facial regions [right (1) and left (2) infraorbital, right (3) and left (4) cheek, subnasal (5), lips (6), and chin (7)]. **c** Baseline facial skin blood flow is compared among the facial regions ($n = 12$ subjects). Skin blood flow in the lips was the highest. *AU* arbitrary unit

Cardiovascular recordings

The cardiovascular responses during exposures to emotionally charged movies were examined in 9 out of the 12 subjects. A pair of electrodes (Magnerode, TE-18M-3, Fukuda Denshi, Tokyo, Japan) and a ground electrode were attached on the chest to measure the electrocardiogram (ECG). The ECG signal and respiratory movement were monitored with a telemetry system (DynaScope DS-3140, Fukuda Denshi, Tokyo, Japan). AP was noninvasively and continuously measured with a Finometer (Finapres Medical Systems BV, Arnhem, The Netherlands) with the cuff attached to the left middle or index finger. The AP waveform was sampled at a frequency of 200 Hz. The beat-to-beat values of mean AP (MAP), HR, cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR) were calculated from the aortic pressure waveform using Modelflow software (BeatScope 1.1, Finapres Medical Systems BV, Arnhem, The Netherlands).

Experimental protocols

Audiovisual stimulation by emotionally charged movies was given to all 12 subjects using the eyeglass type of head-mount goggle display (HMZ-T3, Sony Co., Tokyo, Japan) with a headphone, which was controlled by a computer (Fig. 1). The three kinds of movies were selected as emotionally charged stimulation: a comical movie (called "Manzai") performed by two Japanese comedians, a Japanese horror movie (titled "The Juon" Japanese version Toho, Japan 2000), and a night landscape movie. It was expected that the comical movie might charge the most pleasant feeling, while the horror movie might charge the most unpleasant one, and the landscape movie might be neutral. Each of the emotionally charged interventions (comedy, landscape, and horror) consisted of three consecutive bouts involving different scenes captured from an individual movie. The cardiovascular and skin blood flow data were sampled at each bout, which contained the periods before (for 1 min), during (for 2 min), and after movie stimulation (for 1 min). At the prestimulation period, the subjects watched a small white circle on a black background in the goggles. The data over the 36-s period before the onset of emotional stimulation were regarded as the baseline control. A time interval between bouts in a given emotional intervention was 4 ± 0.2 min. Immediately after the cessation of each bout, questions about the subjective ratings of pleasantness and consciousness were asked. The transit time interval between emotional interventions (for example, from comedy to landscape) was 5 ± 0.5 min. The order of the three movie interventions was randomized. On a separate day, a mental arithmetic test was conducted in 6 of the 12 subjects. The subjects were asked to sequentially subtract 7 from a given three-digit number for a period of 120 s. The data over the 36-s period before a mental arithmetic test were regarded as the baseline control.

Any skin movement due to possible facial expressions (such as smiling and laughing, etc.) during emotional challenges caused an artifact in the laser speckle flow measurement. Therefore, we asked the subjects not to evoke any facial movement during emotional challenges as much as possible. Whenever such facial movement occurred during a trial of the emotional challenges, we could easily notice a big artifact on the laser speckle flow image and remove the data acquired. Thus, the present data collection involved no artifacts due to facial expressions during the emotional challenges. As time control without any emotional stimulation, the baseline changes in facial skin blood flows were measured in five subjects, according to the same experimental protocols. Facial skin blood flows did not change significantly throughout the time control experiments.

Subjective ratings of feelings stimulated during emotionally charged movies

Questions about the subjective feelings of pleasantness and consciousness were asked immediately after each bout of the emotionally charged stimulation, according to previous studies [21–26]. Pleasantness was rated with 11 grades from "the most pleasant" (+5) to "the most unpleasant" (−5). Consciousness was also rated with 11 grades from "the most conscious" (+5) to "the most unconscious" (−5). We explained to the subjects that "the most conscious" means the most awake condition being fully aware of the situation, while "the most unconscious" means the drowsiest or sleepiest condition being fully unaware of the situation. The assessment tables were displayed in the goggles, and the ratings were determined according to the feelings by individual subjects. Since consciousness generally imposes a strong influence on the autonomic nervous system, we assessed the rating of consciousness as well as pleasantness.

Immediately after completing the three consecutive bouts of each emotionally charged movie, we asked the subjects to estimate the more detailed subjective feelings (tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion) for the movie type (comedy, landscape, and horror) based on the responses to a total of 36 questionnaires by a modified Japanese version of the profile of mood states (POMS) [27–32]. The subjects answered each question on the questionnaire with five grades from "not at all" (0) to "the most adequate" (4).

Data and statistical analyses

The data on limb skin blood flows and systemic cardiovascular variables were stored to a computer at a sampling frequency of 1 kHz (PowerLab 16/35, ADInstruments-Japan, Nagoya, Japan) for off-line analysis. The two-dimensional facial speckle flowmetry images were acquired every 12 s, and regional facial skin blood flows were sequentially obtained from seven different facial regions (right and left infraorbital, subnasal, lips, right and left cheeks, and chin) throughout the experiments, as shown in Fig. 1. The original data on facial and limb skin blood flows are expressed as arbitrary units (AU) throughout the text and figures. The zero value of the AU indicated the absolute zero level of skin blood flow. The data on limb skin blood flows and systemic cardiovascular variables during the three consecutive bouts of each emotionally charged movie were combined with the facial skin blood flow data every 12 s. The mean value of each variable obtained over the three consecutive bouts was defined as the response during exposure to a type of emotionally charged movie. With respect to infraorbital and cheek skin blood flows, the bilateral blood flow data were combined together (as shown in Fig. 5), because the time course and magnitude of the responses were similar on both sides. The individual mean values during a type of emotional challenge were further averaged among the subjects.

The systemic cardiovascular changes during an emotional challenge were analyzed by a one-way ANOVA with repeated measures and a Dunnett post hoc test. Cutaneous vascular conductance was calculated as a ratio between skin blood flow and MAP. Then, the values of skin blood flow and vascular conductance were transformed into the relative %changes against the pre-intervention 100% values for every subject. The %responses in facial and limb skin blood flow and vascular conductance during emotional interventions were analyzed by a two-way ANOVA with repeated measures (main effects of time and movie type) and a Holm-Sidak post hoc test to examine whether the %changes during and after emotional interventions were

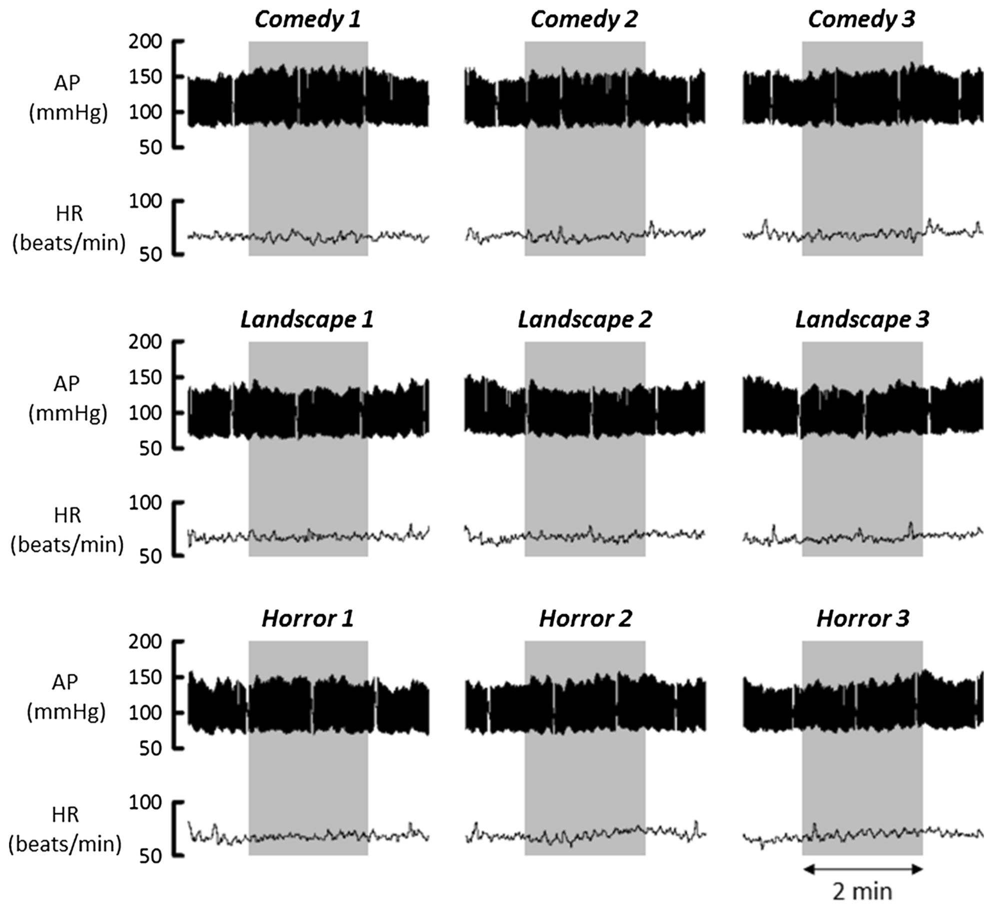


Fig. 2 An example of the changes in arterial blood pressure (AP) and heart rate (HR) during viewing of emotionally charged movies (comedy, landscape, and horror) in a subject

different compared to the pre-intervention control period and among movie types. The subjective ratings of pleasantness and consciousness and the POMS scores were compared among emotionally charged movies by a Friedman signed rank test with a Tukey post hoc test. The relationships between the subjective rating of pleasantness or consciousness and the %changes in facial and limb skin blood flows or the cardiovascular responses were assessed by a Spearman rank correlation method. Furthermore, the correlation between the extents of pleasantness and consciousness was also analyzed by Spearman's rank method. A level of statistical significance was defined at $P < 0.05$ in all cases. All statistical analyses were performed using SigmaPlot®, version 12.5 (Systat Software, San Jose, CA). All variables are expressed as mean ± SE.

Results

Cardiovascular responses during exposure to emotionally charged movies

The resting HR and MAP were 64 ± 3 beats/min and 98 ± 3 mmHg, respectively. The continuous measurements of AP and HR during exposure to emotionally charged movies in a subject are exemplified in Fig. 2. Obviously, AP and HR did not change during exposure to emotionally charged movies. The changes in HR, SV, and MAP were not statistically significant ($P > 0.05$) against the baseline control during exposure to any emotionally charged movie, although CO and TPR slightly changed ($P < 0.05$) during exposure to the landscape or horror movie (Fig. 3a).

Subjective ratings of pleasantness and consciousness

As soon as every bout for an individual movie was completed, questions about the subjective ratings of pleasantness and consciousness in relation to exposure to the emotionally charged movie were asked. As shown in Fig. 3b, the pleasantness score was the highest with the comedy movie and the lowest with the horror movie; the difference in the pleasantness score was significant ($P < 0.05$). On the other hand, the consciousness score was higher ($P < 0.05$) with both comedy and horror movies than for the landscape movie (Fig. 3b). There was no significant correlation between the extents of pleasantness and consciousness [correlation coefficient (γ) = 0.01, $P = 0.947$ by Spearman's rank correlation method].

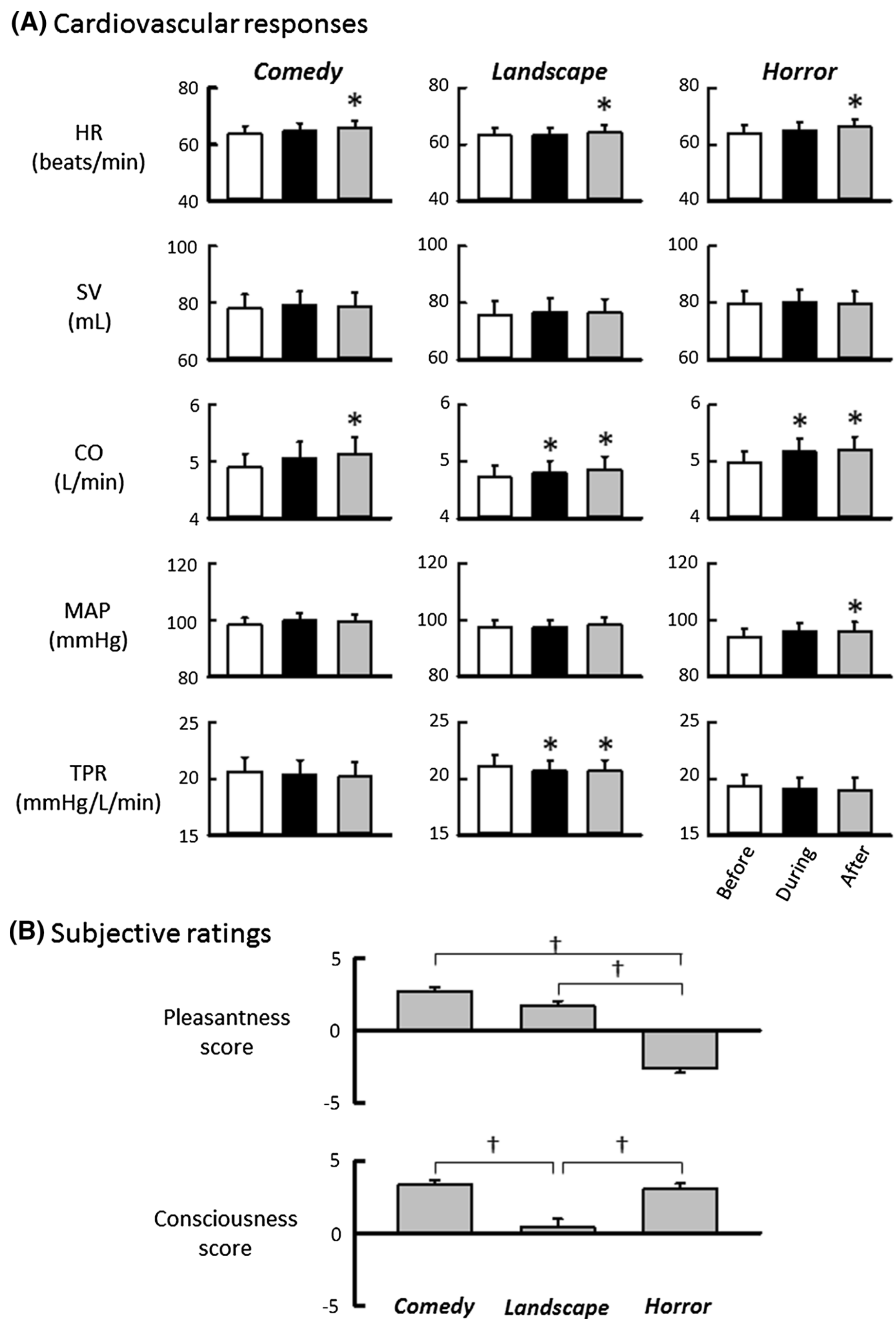


Fig. 3 **a** The responses in HR, stroke volume (SV), cardiac output (CO), mean arterial blood pressure (MAP), and total peripheral resistance (TPR) during emotionally charged challenges (comedy, landscape, and horror) in nine subjects. Each of the emotional interventions consisted of three consecutive bouts involving different movie scenes. The data during each intervention were collected as the mean value over the three bouts, and they were further averaged among the subjects. **b** Comparisons of the subjective ratings of pleasantness and consciousness among emotionally charged movies (comedy, landscape, and horror). The subjective ratings collected immediately after every bout of an emotional challenge were averaged among 12 subjects. *Significant difference ($P < 0.05$) from the baseline control before emotional challenge. † Significant difference ($P < 0.05$) between emotional challenges

After the three bouts for a movie had been completed, more detailed subjective ratings of feelings were requested using the modified POMS questionnaires. Compared to the landscape movie, a feeling of vigor was the highest with the comedy movie and the lowest with the horror movie; the difference between them was significant ($P < 0.05$). Feelings of anxiety, depression, and fatigue were significantly increased ($P < 0.05$) only after exposure to the horror movie.

Responses in facial skin blood flow during exposure to emotionally charged movies

Figure 4 shows an example of the responses in facial skin blood flows during exposure to emotionally charged movies in a subject. Baseline facial skin blood flow was the highest in the lips (Figs. 1, 4). Figure 5 represents the time courses and magnitudes of the average facial skin blood flows, which were obtained during the three bouts of

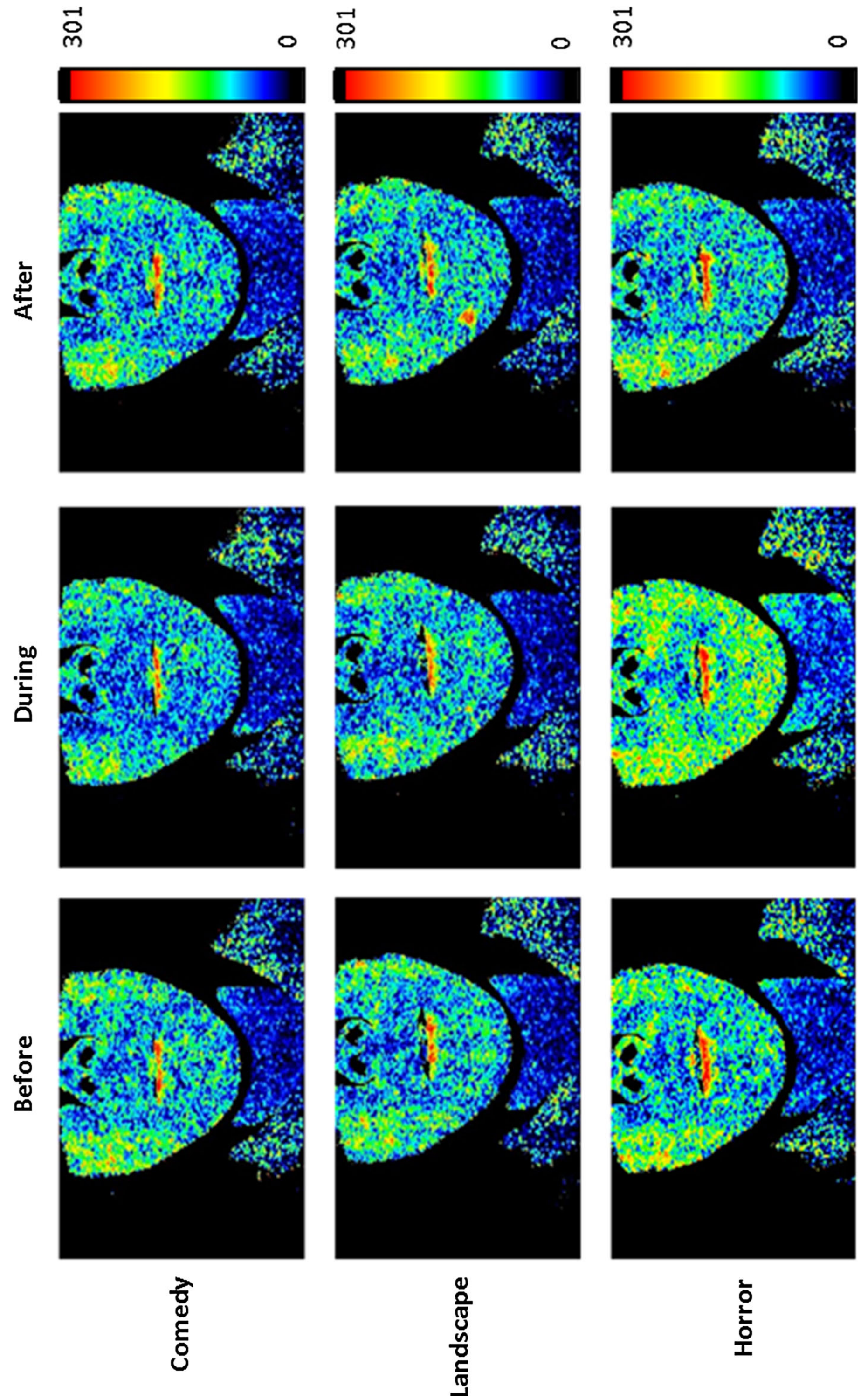


Fig. 4 An example of two-dimensional measurements of facial skin blood flow during exposures to emotionally charged movies (comedy, landscape, and horror) in a subject

individual emotional stimulation. Evidently, during exposure to the comedy movie, facial skin blood flows decreased, especially in the lips (Fig. 5). In contrast, exposure to either a horror or landscape movie failed to cause obvious changes in any facial skin blood flow. The average %changes in regional facial skin blood flows during emotional stimulation are compared against the baseline control and also compared among the emotional interventions in Fig. 6. All facial skin blood flows decreased ($P < 0.05$) from the control during exposure to the comedy movie. However, any facial skin blood flows failed to change significantly during exposure to either the

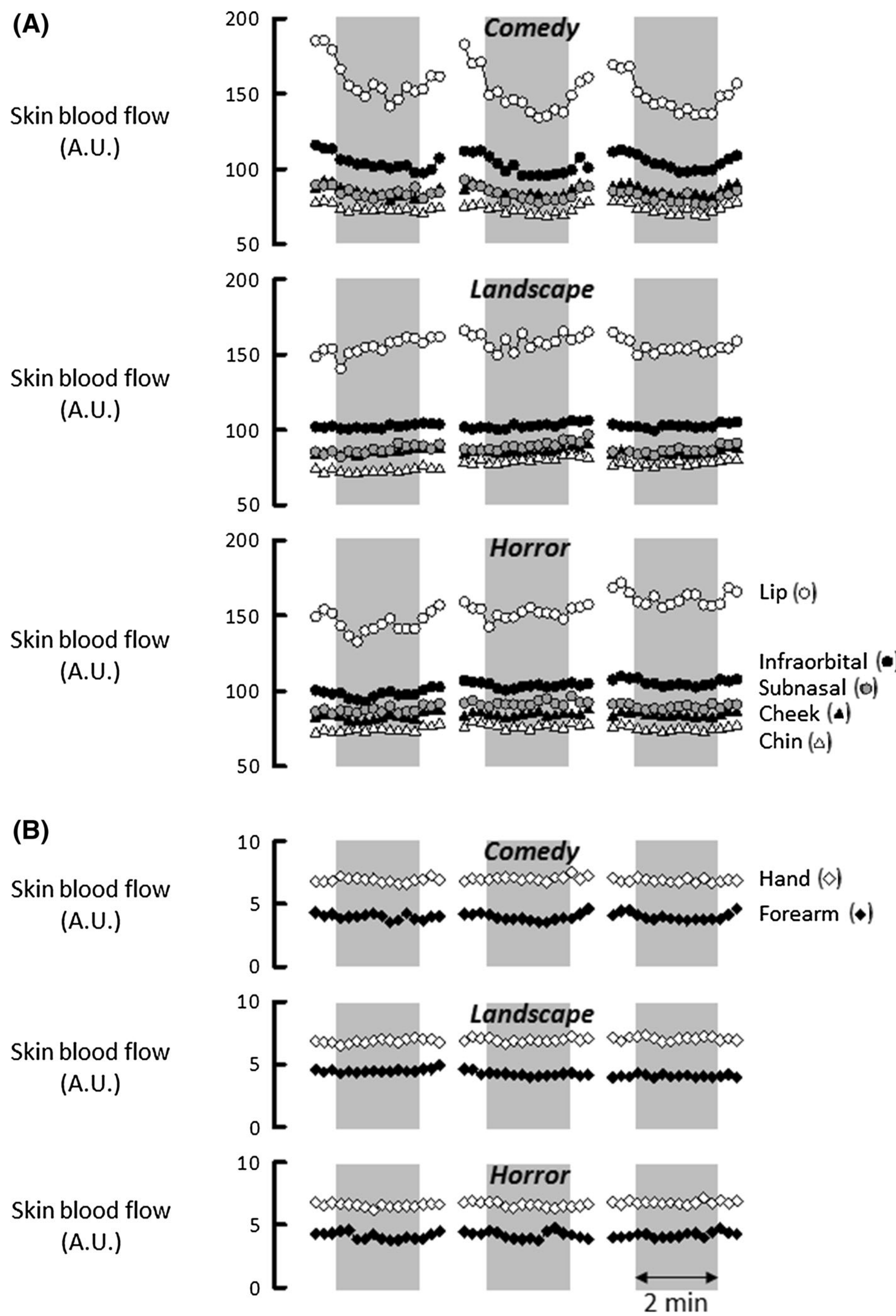
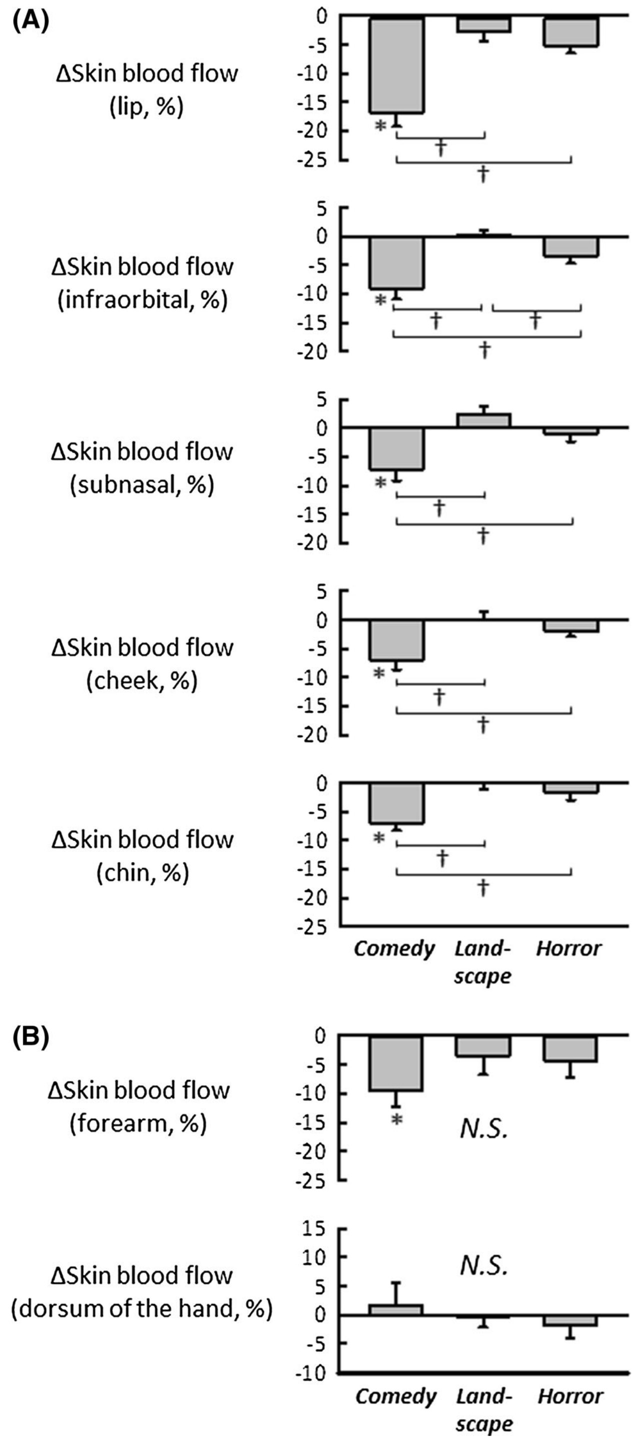


Fig. 5 **a** The time courses of the responses in facial skin blood flows during the three bouts of emotionally charged challenges (comedy, landscape, and horror) in 12 subjects. The data on skin blood flows in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. The skin blood flows in the five facial regions are superimposed. **b** The time courses of the responses in limb skin blood flows (forearm and the dorsum of the hand) when viewing emotionally charged movies (comedy, landscape, and horror) in 6 or 7 of the 12 subjects. *AU* arbitrary unit

horror or landscape movie. When comparing the average %changes in facial skin blood flows among the movies, the response magnitudes in the case of the comedy movie were much greater ($P < 0.05$) than those during exposure to the horror and landscape movies (Fig. 6). The characteristics of the responses in vascular conductance of facial skin regions were almost similar to those of the blood flow responses, although vascular conductance in all facial skin regions decreased ($P < 0.05$) when viewing a horror movie (Fig. 7).

Responses in limb skin blood flow during exposure to emotionally charged movies

The time courses and magnitudes of the average limb skin blood flows (forearm and the dorsum of the hand) during exposure to emotionally charged movies are shown in Fig. 5. The time course data revealed that limb skin blood flows unchanged from the baseline during exposure to any emotionally charged movies. The relative %changes in limb skin blood flows were mostly insignificant during exposure



◀**Fig. 6** **a** The %changes in regional facial skin blood flow during emotionally charged challenges (comedy, landscape, and horror) in 12 subjects. The %change data were obtained compared to the baseline control levels (denoted as 100%) for every subject. The data on skin blood flows in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. The data during each emotional challenge were collected as the mean value over the three consecutive bouts, and they were further averaged among the subjects. **b** The %changes in limb skin blood flow (forearm and the dorsum of the hand) during the emotionally charged challenges were similarly analyzed in 6 or 7 of the 12 subjects. *Significant difference ($P < 0.05$) from the baseline control before emotional challenge. † Significant difference ($P < 0.05$) between emotional challenges. *NS* not significant between emotional challenges

to emotionally charged movies, although forearm skin blood flow slightly decreased ($P < 0.05$) during exposure to the comedy movie (Fig. 6). The characteristics of the responses in limb cutaneous vascular conductance were almost similar to those of the limb blood flow responses, although forearm vascular conductance decreased ($P < 0.05$) when viewing a horror movie (Fig. 7).

Relationships between the subjective ratings and the responses in skin blood flow or vascular conductance

Whether the responses in facial skin blood flow and vascular conductance significantly correlated with the subjective rating of pleasantness or consciousness is clarified in Figs. 8 and 9. With respect to pleasantness, significant correlations ($P < 0.05$) were found with the changes in skin blood flows of the lip, cheek, and chin regions. The changes in skin blood flows in the infraorbital and subnasal regions, however, did not show a significant correlation (Fig. 8). The changes in lip skin vascular conductance alone, but not others, had a significant correlation with pleasantness (Fig. 9). With respect to consciousness, there were significant correlations ($P < 0.05$) with the changes in skin blood flow in all facial regions and with the changes in skin vascular conductance in the infraorbital, subnasal, and chin regions (Figs. 8, 9).

In contrast to the facial regions, limb skin blood flow and vascular conductance (forearm and the dorsum of the hand) had no significant correlations ($P > 0.05$) with the subjective ratings of pleasantness and consciousness (Figs. 8, 9). Moreover, none of the systemic cardiovascular variables (HR, SV, CO, MAP, and TPR) correlated to either pleasantness or consciousness ($P > 0.05$, not shown).

Responses in facial and limb skin blood flows during the mental arithmetic task

Figure 10 summarizes the average responses in facial and limb skin blood flows during a mental arithmetic task. All facial and limb skin blood flows did not change ($P > 0.05$) from the baseline during the mental arithmetic task, although lip skin blood flow tended to decrease by 7.5 ± 8.6% ($P = 0.072$). On the other hand, significant increases in HR (7 ± 2 beats/min) and CO (0.5 ± 0.2 l/min), and MAP (4 ± 1 mmHg) and significant decrease in TPR (1.1 ± 0.5 mmHg/l/min) were observed. Immediately after the mental arithmetic task, the pleasantness

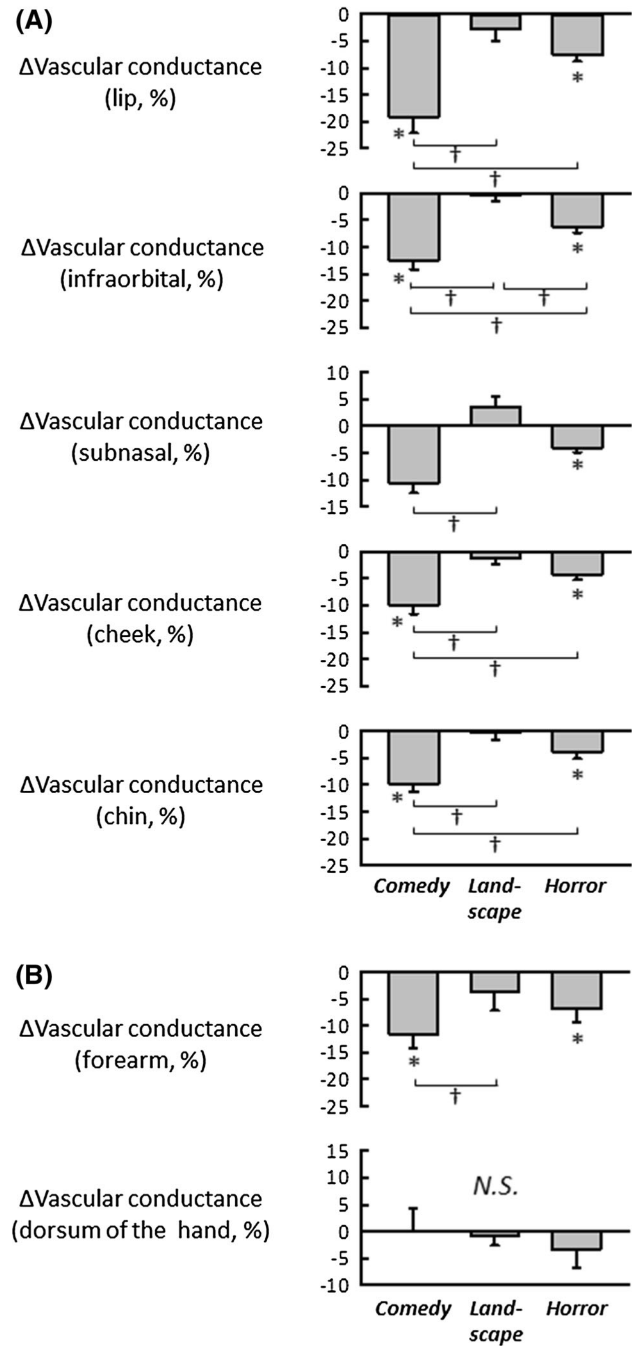


Fig. 7 **a** The %changes in regional facial skin vascular conductance during emotionally charged challenges (comedy, landscape, and horror) in 9 subjects. The data on skin vascular conductance in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. The data during each emotional challenge were collected as the mean value over the three consecutive bouts and were further averaged among the subjects. **b** The %changes in limb skin vascular conductance (forearm and the dorsum of the hand) during the emotionally charged challenges were similarly analyzed in 6 or 7 of the 12 subjects. *Significant difference ($P < 0.05$) from the baseline control before emotional challenge. †Significant difference ($P < 0.05$) between emotional challenges. *NS* not significant between emotional challenges

score was rated as -0.4 ± 0.4 and the consciousness score as 2.6 ± 0.3, suggesting that the pleasantness level seemed neutral, while the conscious level was close to the level during the comedy and horror stimulation.

Discussion

For the first time to our knowledge, the present study measured the changes in regional facial skin blood flows during audiovisually elicited emotional challenges by using noninvasive two-dimensional laser speckle flowmetry. Limb skin blood flows (forearm and the dorsum of the hand) and systemic cardiovascular variables were simultaneously measured. We hypothesized that a positively charged emotional stimulus may decrease facial skin blood flows as opposed to a negatively charged emotional stimulus such as anger or embarrassment. The major new findings of this study are (1) that positively charged emotional stimulation decreased regional facial skin blood flow (especially in the lips), whereas negatively charged emotional stimulation did not alter facial skin blood flow as opposed to our expectation; (2) that although regional facial skin vascular conductance decreased during exposure to comedy and horror movies, the reduction in vascular conductance was much greater in the case of a comedy movie; (3) that the relative %changes in skin blood flow of most facial regions were inversely correlated with the subjective rating of pleasantness or consciousness; (4) that the relative %changes in lip skin vascular conductance had an inverse correlation with the subjective rating of pleasantness, while the %changes in infraorbital, subnasal, and chin skin vascular conductance had an inverse correlation with the subjective rating of consciousness; (5) that none of the changes in systemic cardiovascular variables and limb skin blood flow and vascular conductance correlated with the subjective ratings of pleasantness and consciousness. Taken together, it is suggested that the more positive emotional status becomes, the more neurally mediated vasoconstriction may occur in facial skin blood vessels without changing the limb skin blood flows and systemic cardiovascular variables.

Facial skin blood flow as a physiological estimate of emotional and/or mood status

In this study, we found for the first time significant decreases in all facial skin blood flows when viewing a positively charged movie, some of which had significant correlations with the subjective rating of pleasantness. Although the subjective rating against the horror movie revealed a clear unpleasant feeling, the responses in facial

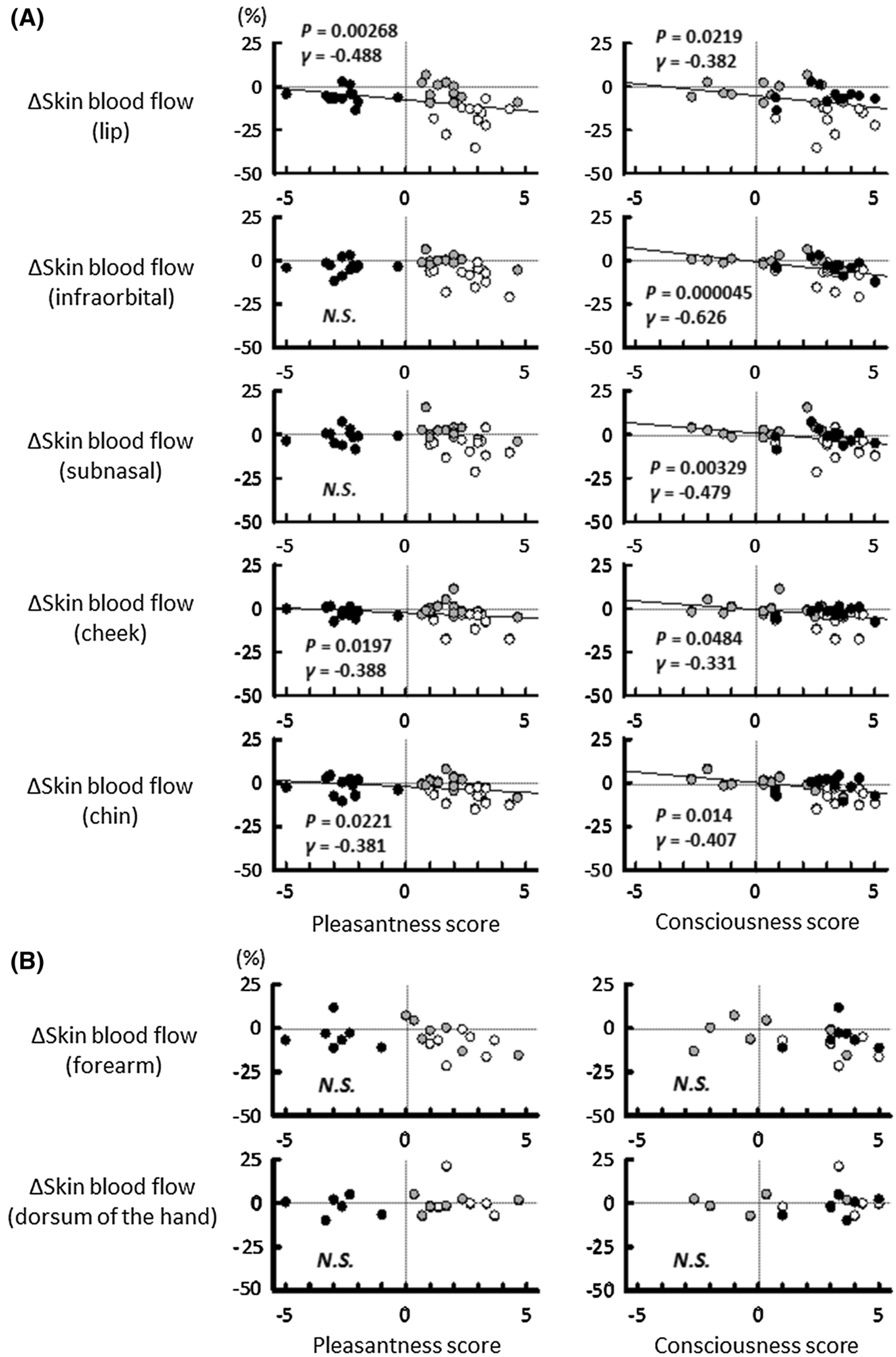


Fig. 8 **a** The relationships between the %changes in facial skin blood flows and the subjective ratings of pleasantness or consciousness based on the data obtained from 12 subjects. The data on skin blood flows in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. **b** The relationships between the %changes in limb skin blood flows and the subjective ratings of pleasantness or consciousness based on the data obtained from six or seven subjects. If a significant correlation was obtained, a linear regression line was inserted. The data points obtained during exposures to emotionally charged movies are represented by different symbols [comedy (*white circles*), landscape (*grey circles*), and horror (*black circles*)]. *NS* not significant. The *P* values and correlation coefficients (γ) by Spearman's rank correlation method were inserted in all significant comparisons ($P < 0.05$)

skin blood flow showed an increase in some subjects (as exemplified in Fig. 4) but not in others. Accordingly, facial skin blood flows during negatively charged emotional stimulation failed to show a significant response from the baseline (Fig. 6), like forehead skin blood flow [6]. The reason for this was that the responses of facial skin blood flows during exposure to a horror movie had great inter-subject variation, despite less variation of the subjective ratings. At least two factors may relate to the intersubject variation. First, some subjects expressed more complex feelings for the horror movie compared to the comedy or landscape movie. The horror movie caused partly neutral or

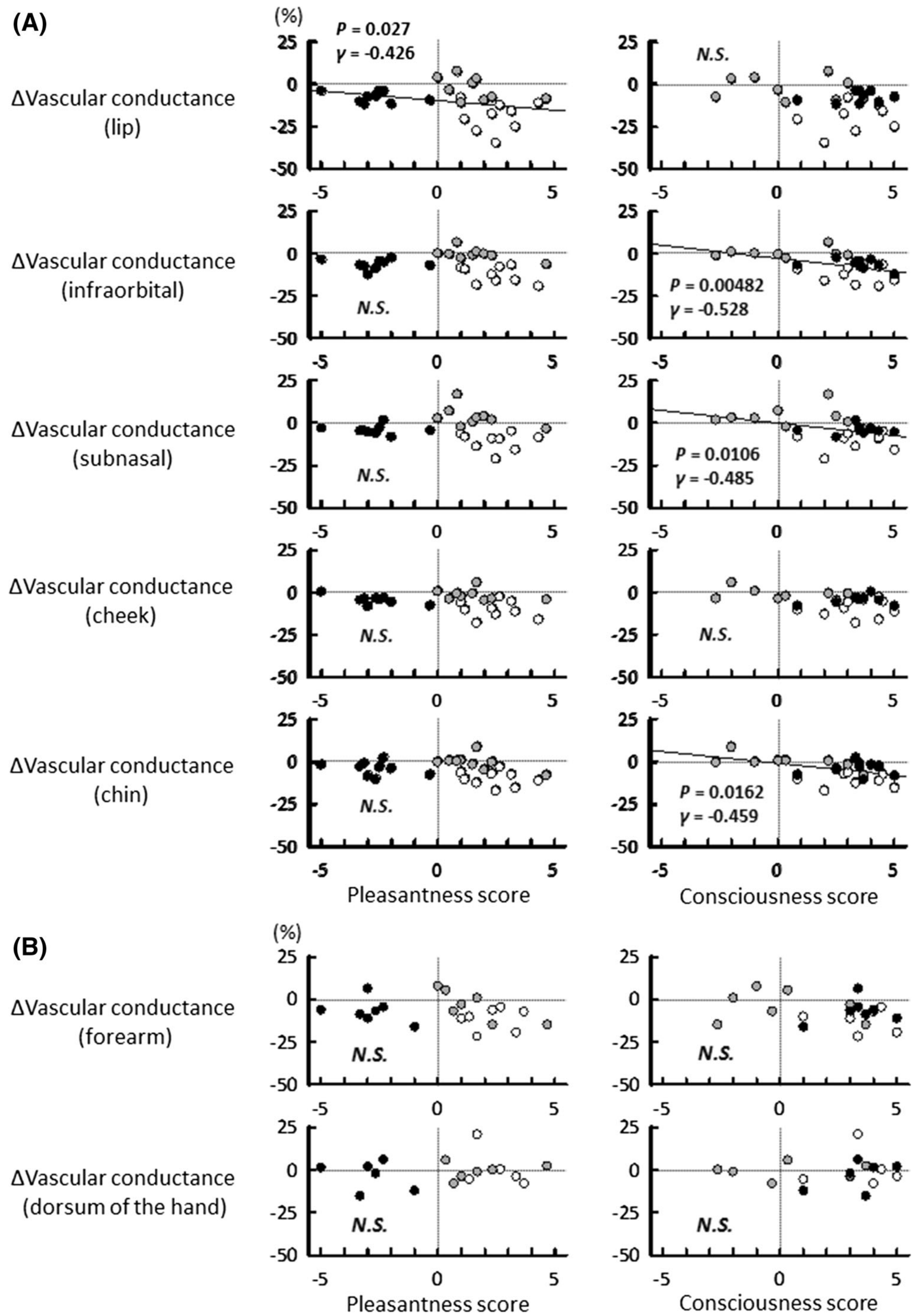


Fig. 9 **a** The relationships between the %changes in facial skin vascular conductance and the subjective ratings of pleasantness or consciousness based on the data obtained from 9 subjects. The data on skin vascular conductance in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. **b** The relationships between the %changes in limb skin vascular conductance and the subjective ratings of pleasantness or consciousness based on the data obtained from six or seven subjects. If a significant correlation was obtained, a linear regression line was inserted. The data points obtained during exposures to emotionally charged movies are represented by different symbols [comedy (*white circles*), landscape (*grey circles*), and horror (*black circles*)]. *NS* not significant. The *P* values and correlation coefficients (γ) by Spearman's rank correlation method were inserted in all significant comparisons ($P < 0.05$)

positive feelings depending on the subjects, which made it difficult to detect a significant response of facial skin blood flow. Second, the comedy and landscape movies may impose relatively stationary stimuli, while the horror movie may not be stationary and may change every moment, making somewhat variant responses in facial skin blood flows (as discussed later as one of the limitations). However, the time course data on facial skin blood flows did not show any obvious temporal changes in each bout (as shown in Fig. 5). As a result, the finding suggests that the present negatively charged emotional stimulation did not evoke a significant response in facial skin blood flows, although it cannot be neglected that the facial skin blood flow response, if any, may be masked by the great intersubject variation.

It is likely that facial skin blood flow reflects emotional changes with a higher sensitivity than limb skin blood flow and systemic hemodynamic variables. The decreases in

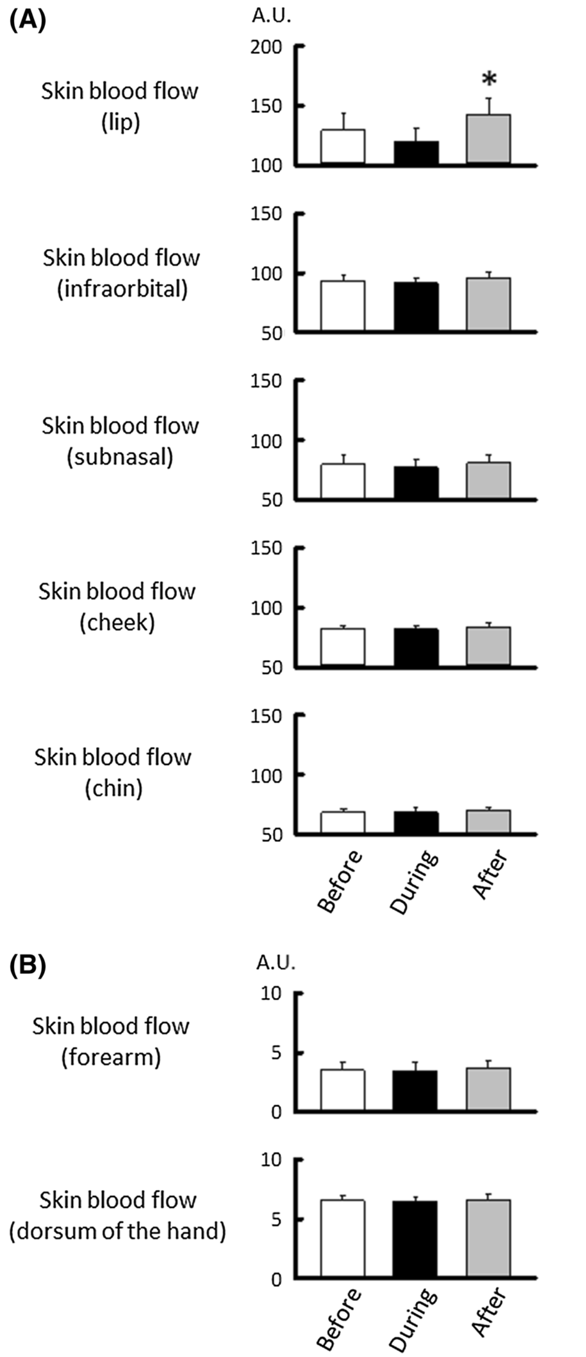


Fig. 10 **a** The responses in facial skin blood flows during the mental arithmetic task in six subjects. The data on skin blood flows in the bilateral infraorbital and cheek regions were pooled and averaged, respectively. **b** The responses in limb skin blood flows (forearm and the dorsum of the hand) during the mental arithmetic task in the same subjects. *Significant difference ($P < 0.05$) from the baseline control before the mental arithmetic task. *AU* arbitrary unit

facial skin blood flows during exposure to the positively charged movie occurred without any significant changes in MAP (Figs. 2, 3), suggesting that the decreased blood flow was not passively evoked by a decrease in perfusion pressure but was actively induced by the autonomic nervous system. This notion is supported by the significant decreases in facial skin vascular conductance during exposure to the positively charged movie (Fig. 7). Sympathetic postganglionic nerve fibers run along branches of the external and internal carotid arteries and terminate in facial skin blood vessels. Activation of the facial sympathetic nerve may cause α-adrenergic vasoconstriction of cutaneous blood vessels [9]. On the other hand, facial parasympathetic preganglionic fibers originating from the brain stem contact postganglionic neurons at the otic and pterygopalatine ganglia, whose axons terminate in facial skin blood vessels in the lips [10]. Activation of the parasympathetic nerve may cause vasodilatation in facial cutaneous blood vessels [11]. Accordingly, the decrease in facial skin blood flow evoked by positively charged emotional stimulation may be caused by an increase in sympathetic vasoconstrictor nerve activity and/or a decrease in parasympathetic vasodilator nerve activity. It is of interest that facial skin blood flow does not alter during the mental arithmetic test, despite the significant systemic cardiovascular responses that are controlled by the sympathoadrenal activity.

Different regional responses in facial skin blood flow

It is known that the response in facial skin blood flow shows great regional differences in many circumstances during thermoregulation with body heating [12, 14, 15], a cold pressor test [33], static handgrip exercise [33], irritant chemical stimulation in the oral cavity [34], and painful tooth stimulation [18]. In this study, skin blood flow in all facial regions decreased during exposure to the comedy movie. The decrease of skin blood flow in the lip, cheek, and chin regions had significant correlation with the subjective rating of pleasantness (Fig. 7), while lip skin vascular conductance alone had a significant correlation with pleasantness (Fig. 8). These findings may lead to the assumption that a specific pattern of the changes in regional facial skin blood flows gives insight into identification of an emotional or mood status.

Limb skin blood flow and systemic hemodynamics do not reflect emotional status

In a thermo-neutral condition, sympathetic vasoconstrictor fibers, but not vasodilator fibers, are active in the limb cutaneous vascular bed and the changes in the sympathetic nerve activity may regulate limb cutaneous vascular resistance and blood flow. To assess an emotional or mood status quantitatively in terms of the changes in systemic cardiovascular variables and limb skin sympathetic nerve activity, correlations with a subjective emotional status have been analyzed. In response to fear-induced stress, it has been reported that HR and MAP increased but limb skin blood flow and surface temperature decreased in humans and conscious animals [5, 6]. In addition to the emotional stimulation, mental stress itself and/or stress-induced defensive behavior may cause the characteristic cardiovascular response and cutaneous vasoconstriction [1–4]. Contrary to the data, Brown et al. [7] reported that exposure to emotionally charged pictures did not accompany such cardiovascular response and finger cutaneous vasoconstriction. In this study we found no significant changes in hand skin blood flow and vascular conductance during any emotional challenge, although forearm skin blood flow and vascular conductance decreased during exposure to comedy and/or horror movies (Figs. 6, 7). Furthermore, the systemic cardiovascular variables were not influenced by exposure to the comedy movie, while a slight increase in CO and a slight decrease in TPR were observed during exposure to landscape and/or horror movies (Fig. 3). Taken together, it is difficult to identify an emotional or mood status in terms of the changes in systemic cardiovascular variables and limb skin blood flow and vascular conductance.

Dissimilarity in autonomic control of facial and limb skin blood vessels

Dissimilarity between the responses in facial and limb cutaneous blood vessels has been recognized. Drummond and Quah [16] reported that with anger condition (describing anger-provoking incidents), forehead pulse amplitude increased compared to a neutral condition, whereas finger pulse amplitude decreased. Their finding indirectly suggested that, in response to a negatively charged emotional intervention, forehead cutaneous blood vessels may dilate, whereas finger cutaneous blood vessels may constrict. Kemppainen et al. [18] reported that painful tooth stimulation increased lip skin blood flow but decreased nose and finger skin blood flow. Kashima et al. [33] reported that facial skin blood flow in the forehead, eyelid, cheeks, and lips increased during the cold pressor test, whereas finger skin blood flow decreased during the test. In agreement with these previous findings, we found dissimilarity between the responses in facial and limb cutaneous blood vessels. When skin blood flow and vascular conductance of all facial regions decreased during exposure to a positively charged emotional intervention, hand skin blood flow and vascular conductance did not change significantly although the variables of the forearm skin area decreased (Figs. 5, 6, 7). Furthermore, the changes in skin blood flow and vascular conductance of some facial regions had significant correlations with the subjective ratings of pleasantness and consciousness, whereas limb skin blood flow and vascular conductance had no significant correlations with the subjective ratings (Figs. 8, 9). The dissociation clearly indicates that autonomic regulation of cutaneous blood vessels is different between the facial and limb regions. This notion is conceivable because it is known that sympathetic nervous activity is non-uniform among organs and controlled depending on a physiological function specific for a given organ [35].

Limitations

Several substantial limitations are involved in this study. First, the data on skin blood flow in the upper part of the face (including the forehead and eyelids) were lacking because of a technical limitation in this study. Since skin blood flows in the forehead and eyelid regions increased in response to emotional embarrassment or chemical taste stimulation [16, 17, 34], it is expected that the skin blood flows would respond to the present emotional interventions. Second, the two-dimensional facial skin blood flow images were obtained by scanning the lower part of the face with the line-sensing device, and the time interval between the first and last scanned data on a given skin blood flow image was $\sim$12 s. Thus, the simultaneous comparison of the skin blood flow data was not allowed in this study. Third, although the comedy and night landscape movies provide relatively stationary audiovisual stimulation over the elapsed time period, the horror movie consisted of many variant scenes containing not only fear and negative feeling but also neutral or positive feeling. Thus, the audiovisual stimulation by the horror movie seemed not always to be stationary and constant. For this reason, the responses in facial skin blood flow became variable among subjects and even in a given subject. To avoid the nonstationary effect of the horror movie, we should use negatively charged static pictures taken from the database of the international affective picture system in the future [36]. The data obtained by stimulation of the emotionally charged pictures may provide a clear dissociation in facial skin blood flow between negative and positive feelings.

Conclusions

The present study has examined for the first time the changes in regional facial skin blood flows in response to audiovisually elicited emotional challenges. Regional facial skin blood flows, especially in the lips, decreased while viewing a positively charged movie. The decreases in the facial skin blood flows correlated with the subjective ratings of pleasantness and consciousness, whereas none of the changes in limb skin blood flows and systemic hemodynamics correlated with them. Therefore, it is suggested that the changes in facial skin blood flows may serve as a more sensitive tool to assess an emotional or mood status.

Acknowledgements This study was supported by the Center of Innovation (COI) Program from the Japan Science and Technology Agency (JST) and partly supported by Grants-in-Aid (no. 15H03061) for Scientific Research (B) from the Japan Society for the Promotion of Science.

Compliance with ethical standards

Funding This study was funded by the Center of Innovation (COI) Program from the Japan Science and Technology Agency (JST) and partly supported by Grants-in-Aid (no. 15H03061) for Scientific Research (B) from the Japan Society for the Promotion of Science.

Conflict of interest All authors declare that we have no conflict of interest about this study.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study prior to the experiments.

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