Effects of second flush Darjeeling tea aroma on psychological and nervous system activities

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The purpose of this study was to clarify the psychophysiological effects of second flush Darjeeling tea aroma. First, the preference for second flush Darjeeling tea aroma and the impressions of the aroma were evaluated. Next, an integrative physiological evaluation of autonomic and central nervous system activities was performed along with psychological evaluation. Inhalation of second flush Darjeeling tea aroma significantly reduced “subjective stress,” “depression/anxiety,” and “hostility.” “Invigoration” and “inactivity” increased significantly, suggesting a sedative effect on psychology. Physiologically, the miosis rate and peripheral skin temperature increased significantly after inhalation of second flush Darjeeling tea aroma, suggesting that sympathetic nerve activity was suppressed and parasympathetic nerve activity became dominant. Furthermore, a significant decrease in blood flow was observed in the prefrontal area of the brain, suggesting a sedative effect on central nervous system activity. While second flush Darjeeling tea aroma had a sedative effect on the psychophysiological level, the physiological effects on the autonomic nervous system activity was not correlated with the preference of the aroma or its psychological effects.

1. Introduction

Black tea, along with coffee and cocoa, is one of the world’s three most palatable beverages, and tea is the second most popular drink in the world after mineral water. Black tea attracts people with its exquisite aroma unique to fermented tea. Tea drinking started in the late 17th century with the elegant custom of tea parties for socializing mainly among British noblewomen, and in modern times, black tea has become more readily available for casual and convenient lifestyles, such as tea breaks at workplaces. Black tea has always served as a tool for communication and as a luxury item that enriches our lives by helping to change the mood and relax. Black tea and other palatable beverages contain various bioactive components, which are believed to affect mood and emotion by acting on receptors in the brain1. For example, ethanol contained in alcoholic beverages2 and caffeine contained in tea and coffee3 are well known bioactive ingredients of palatable beverages. In recent years, there has been a growing interest in the aroma unique to each palatable beverage as one that contributes to physical and mental health. The reported psychological and physiological effects of the aroma of palatable beverages include the sedative effect of the aroma of jasmine tea4 and beer5, and the stress-reducing effect of the aroma of whiskey6 and coffee7.

Regarding the psychophysiological and physiological benefits of black tea aroma, it has been reported that psychological tension and anxiety and chromogranin A, a salivary stress marker, are reduced by sniffing the aroma of black tea in stressed conditions8. However, the effects of black tea aroma on the neuronal activity of the autonomic nervous system and central nervous system in a normal state under non-stress conditions have not yet been elucidated.

While research has been conducted on the psychological and physiological effectiveness of aromas, several challenges have been raised in conducting such research. These challenges include differences in the olfactory properties of aromas and the methods of evaluation9〜11. Specifically, there are individual differences in olfactory sensitivity, sensory thresholds, cognition, and memory for aromas, as well as a placebo effect due to psychological influences such as prefer-
ence and pleasure-unpleasure for specific aromas. Studies suggest that sex, age, and region contribute to such differences. There are two mechanisms by which aromas are perceived. One is the neurological pathway, in which aroma compounds act on olfactory receptors and are transmitted to the cerebral cortex where they are recognized as aroma via olfactory cells and the olfactory bulb. The other pathway is the pharmacological pathway, in which aroma compounds are transmitted directly to the brain and organs through the olfactory mucosa and blood circulation from the lungs. In the neurological transmission pathway, psychological factors are considered to have an influence since the aroma is recognized via the olfactory cortex including the partial corpus callosum, thalamus, orbitofrontal cortex, hypothalamus, frontal cortex, and hippocampus. When examining the effectiveness of aromas using samples, such as essential oils and food, it is possible that the study subjects may have positive or negative orientation attitudes depending on their preferences and perceptions of aromas since they may already have prior knowledge and ideas about the aroma. In order to address such placebo effects, it is necessary to examine the attributes of the study subjects beforehand, as well as the relationship between their preference for the study sample and their psychological effects of the aromas.

As for the methodological aspects, heart rate variability analysis, which is an indicator of autonomic nervous system activity, is an effective method for stress evaluation tests to assess the state of increased sympathetic nerve activity, but it is not suitable for evaluation and analysis of the state in which the parasympathetic nerve activity is dominant. As the behavior of the heartbeat is affected by blood pressure and respiratory variations, there are many points to be noted in experiments, such as the need for respiratory control by means of a metronome when inhaling aromas. In recent years, as a simpler and more accurate method of evaluating autonomic nervous system activity, an integrative physiological evaluation method has been proposed in which pupillary light reaction reflecting sympathetic activity, peripheral skin temperature reflecting sympathetic activity, and near-infrared spectroscopy reflecting central nervous system activity are all measured in the same test. The integrative physiological evaluation has revealed that the odors of cedrol, an aroma component derived from coniferous forests, and damask rose have a sedative effect on autonomic and central nervous system activities.

In the present study, we examined the psychological and integrative physiological effects of second flush Darjeeling tea aroma in order to determine its effects under normal, non-stress conditions. We also examined how the preference and psychological effects of second flush Darjeeling tea aroma elicit physiological effects on the activities of the autonomic nervous system.

2. Materials and Methods

2.1 Study sample selection

Taking into account the influence of preference on the psychological and physiological effects of aromas, we investigated the preference for the aroma of commercially available black tea in advance for the purpose of selecting study samples. Seventeen volunteers subjectively evaluated their preference by sniffing the aroma of extracts of second flush Darjeeling tea (SFDJ; second-picked black tea produced in Darjeeling in the Himalayan foothills in the northeast of India), Assam tea (AS; black tea produced in Assam in the southeast of the Himalayas in India), Uva tea (UV; black tea produced in Uva in the highlands of Sri Lanka), and Earl Grey tea (EG; black tea flavored with bergamot essential oil), all of which had different aromas. Aroma preference was evaluated using a 100-mm visual analogue scale (VAS) ranging from 0 (minimum preference) to 100 (maximum preference), and black tea with moderate to high preference was selected as the study sample.

2.2 Study sample preparation

For preparation of the study samples, extraction from 10 g of black tea leaves was performed using 600 mL of boiling mineral water at 100°C. Referring to the recommended extraction method for each characteristic, such as the shape of the tea leaves, we set the extraction time to 4 minutes for SFDJ and EG, 3 minutes for AS, and 2 minutes for UV. Black tea extract (150 mL) was placed in a cup (capacity: 280 mL) with a flap with a high heat retention capacity, and the temperature was maintained at 80 to 85°C. In this study, Darjeeling tea was used as the study sample because it was the most preferred among the four
types of commercially available black tea in the preference investigation. Plain hot water at the same temperature as the study sample was used as a control.

2.3 Subjects

This study enrolled 18 healthy women (aged 32.8 ± 5.0 years). We limited the study subjects to Japanese women around 30 years old because there were concerns that different sensitivity to aromas depending on sex\textsuperscript{12} and age\textsuperscript{13} could have an influence on the psychological and physiological effects. It was confirmed in advance that the subjects had no aversion to black tea, no smoking habit, no sensitivity to cold, no current medication, and no skin disease symptoms such as atopic dermatitis. They were also instructed to get at least 7 hours of adequate sleep the night before the study, to refrain from irritating foods before the study, to limit the intake of caffeine-containing beverages, and not to wear scented cosmetics on the day of the study.

2.4 Ethics statement

This study was performed in conformance with the code of ethics as stipulated in the Declaration of Helsinki, after explaining the study content and methods and obtaining written consent from the subjects. The ethical review was outsourced to contract research organization Chiyoda Paramedical Care Clinic (CPCC) by study sponsor Mitsui Norin Co. Ltd. under approval of the study protocol and agreement on data publication rights, and CPCC executed this study after review and approval by their Ethics Committee (ethics committee approval number: MTN17C1). The study was also registered in advance with the University Hospital Medical Information Network Center (UMIN study ID: UMIN000026670).

2.5 Experimental procedure

The measurement room, which was a private room with windows and doors closed to maintain silence, was set at a room temperature of 23°C, a relative humidity of 40%, and an illuminance of 300 lux. The area of the measurement room where the integrative physiological evaluation was performed was 23.6 m\textsuperscript{2} for pupillary light reaction, peripheral skin temperature, and near-infrared spectroscopy. Pupillary light reaction and peripheral skin temperature were measured after inhalation of the aroma of the study sample or the control sample over 2 minutes following a 2-minute rest. Measurement using near-infrared spectroscopy was performed continuously during the inhalation of the aroma of the study sample or the control sample over 2 minutes following a 2-minute rest. A resting period of 2 minutes was provided between the measurements for the study sample and the control sample.

The study sample and the control sample were placed about 3 cm below the nose for inhalation while the subjects were seated. At the time of measurement, the flap of the cup (1 cm long × 2 cm wide opening) was opened and the subjects inhaled the aroma from the opening for 2 minutes. They were instructed in advance to intuitively fill out the questionnaires and not to ponder over each question. The integrative physiological evaluation was performed sequentially in three measurement rooms for pupillary light reaction, peripheral skin temperature, and near-infrared spectroscopy. Pupillary light reaction and peripheral skin temperature were measured after inhalation of the aroma of the study sample or the control sample over 2 minutes following a 2-minute rest. A resting period of 2 minutes was provided between the measurements for the study sample and the control sample.

2.6 Measurements

2.6.1 Subjective evaluation of aroma preference and impressions

Aroma preference and impressions were evaluated after inhalation of the aroma of the study sample. Subjective preference for aroma was evaluated using a 100-mm VAS\textsuperscript{21,22} ranging from 0 (minimum preference) to 100 (maximum preference). Regarding the impressions of aroma, subjective impressions were evaluated
on the following 11 items using a 100-mm VAS ranging from 0 (minimum impression) to 100 (maximum impression): “elegant,” “relaxing,” “comforting,” “refreshing,” “motivating,” and “mood enhancing” (items related to positive impressions) and “brilliant,” “fresh,” “fruity,” “sweet,” and “plant-like” (items related to aroma characteristics).

2.6.2 Subjective mood scale
Subjective mood was evaluated before and after inhalation of the study sample. Seven evaluation items were employed: “general fatigue,” “subjective stress,” and “boredom” (negative emotions) and “clear headedness,” “concentration,” “motivation,” and “invigoration” (positive emotions). Subjective mood was evaluated using a 100-mm VAS ranging from 0 (minimum mood) to 100 (maximum mood) (mood VAS score).

2.6.3 Multiple mood scale
Subjective emotional states were evaluated before and after inhalation of the aroma of the study sample using the short version of the multiple mood scale (MMS). The MMS consists of 40 questions with the following 8 subscales: “depression/anxiety,” “hostility,” and “fatigue” (negative emotions); “activity,” “inactivity,” and “affinity” (positive emotions); and “focus” and “surprise” (neutral emotions). The degree to which the current emotional condition was applicable was rated on a 4-point scale: “I do not feel it” (1 point), “I hardly feel it” (2 points), “I feel it slightly” (3 points), and “I clearly feel it” (4 points). The scores of 40 items were classified into the 8 subscales, and the total score obtained by simply adding scores for each subscale (MMS score) was evaluated.

2.6.4 Pupillary light reaction
Pupillary light reaction reflecting autonomic nervous system activity was measured using Iriscorder Dual C10641 (Hamamatsu Photonics, Shizuoka, Japan), a goggle-type electronic pupillometer. The subjects were subjected to dark adaptation for 2 minutes in a light-shielded condition with measurement goggles, followed by 0.1-second light stimulation with a red LED. The response of the pupil to light stimulation was measured for 5 seconds, and the percentage of pupil diameter contraction after light stimulation (miosis rate) was determined. After the first 2 minutes of rest, the aroma of the control or study sample was inhaled for 2 minutes, and the pupil response was measured. Based on the results thus obtained, we compared miosis rates.

2.6.5 Peripheral skin temperature
Peripheral skin temperature, which reflects the tonus of sympathetic nerve activity, was measured using a temperature logger LT-8A (Gram, Saitama, Japan). A temperature sensor was attached to the middle of the right index finger of each subject. After 2 minutes of rest, the aroma of the control or study sample was inhaled for 2 minutes, and the skin temperature in the middle of the index finger was measured continuously for 20 seconds. Based on the results thus obtained, we compared the changes in fingertip skin temperature.

2.6.6 Near infrared spectroscopy
The concentration of oxygenated hemoglobin (Oxy-Hb), which indicates activity in the prefrontal area of the brain, was measured using Spectratech OEG-16 (Spectratech, Tokyo, Japan), a brain function measurement device based on near-infrared spectroscopy (NIRS). As shown in Fig. 1, six irradiation probes (light source sensors) and six detection probes (light-receiving sensors) were arranged in six horizontal rows by two vertical rows at 3-cm intervals, with a 16-channel probe holder being used as a measurement point. Two near-infrared wavelengths, 840 nm and 770 nm, were used to continuously measure the Oxy-Hb concentration (mM/mm) at 0.1-second intervals. The Oxy-Hb concentration was calculated based on the modified Lambert-Beer’s law. Since body movements and emotional changes may affect the heart rate, respiration, and blood pressure, we took care to keep the subjects in a resting state and posture, and to maintain silence during measurements. A probe holder was attached to the front of the subject’s head. They were instructed to rest in a sitting position, look at a cross mark at eye level on a wall 2 m in front of

![Fig. 1 Location of sensors and channels in the probe holder for near-infrared spectroscopy](image-url)
LD1-16: irradiation probes (light source sensors); PD1-16: detection probes (light-receiving sensors); CH1-16: 16 channels for measurement.
them, maintain a resting posture without moving their limbs, head, or body, and maintain normal breathing. After 2 minutes of rest, the aroma of the control or study sample was inhaled over 2 minutes, and measurement was performed continuously. The second half of the 2 minutes during which the aroma of the control or study sample was inhaled was used for the analysis of NIRS measurement data. The Oxy-Hb concentrations during the second half of inhalation were averaged for each measurement channel of each subject, and were compared. The baseline was corrected so that it was 0 at the beginning of the measurement for both the control sample and the study sample. Noise and artifacts originating from scalp blood flow were eliminated by hemodynamic modality separation method.

2.7 Statistical analysis

Unless otherwise specified, data are presented as mean ± standard error of the mean (SEM). Subjective evaluation data for preference and impressions were presented as mean ± standard deviation (SD). For the comparison of the mean values of preference for the aroma of the study sample, paired one-way analysis of variance (ANOVA) was employed to verify significance, and Tukey’s method was used for multiple comparisons. The evaluation criteria for the effect size (η²) in one-way ANOVA were η² = 0.14 for a large effect size, η² = 0.06 for a medium effect size, and η² = 0.01 for a small effect size. Paired t-test (two-tailed test) was used to compare the mean values between the two conditions (study sample vs. control sample). The evaluation criteria for the effect size (r) in paired t-test were r = 0.50 for a large effect size, r = 0.30 for a medium effect size, and r = 0.10 for a small effect size. Correlations between variables in the measurement data were analyzed using Pearson’s product-moment correlation coefficient. In the correlation analysis, the mood VAS score and the MMS score were analyzed by subtracting the value before inhalation of the aroma of the study sample from the value after inhalation, while the miosis rate, fingertip skin temperature, and Oxy-Hb concentration were analyzed by subtracting the value after inhalation of the aroma of the control sample from the value after inhalation of the aroma of the study sample. All data were analyzed using the statistical analysis software SPSS Statistics 25 (IBM, Armonk, NY, USA). P values of less than 0.05 were considered statistically significant. A trend toward significance was considered present when the P value was between 0.06 and 0.10.

3. Results

3.1 Study sample selection based on the investigation of preference for black tea aroma

The preference for the aromas of the four types of black tea was evaluated on a VAS scale from 0 to 100: SFDJ: 86.1 ± 2.6, mean ± SD; AS: 70.1 ± 4.1; UV: 61.8 ± 3.7; and EG: 57.6 ± 6.7. Comparison of the differences in preference for the aromas of black tea based on one-way ANOVA showed the effect of preference for black tea aromas was significant [F (3, 64) = 7.72, p = 0.0002, η² = 0.27]. Tukey’s multiple comparison revealed a trend toward significance between SFDJ and AS (p = 0.069) and significant differences between SFDJ and UV (p = 0.002) and between SFDJ and EG (p = 0.0002). We selected SFDJ as the study sample in the present study because it was most preferred among the four types of black tea.

3.2 Preference and impression of SFDJ aroma

Fig. 2 shows the results of the evaluation of preference for SFDJ aroma and its impressions. High scores were noted for “preference” (81.2 ± 3.5, mean ± SD), “elegant” (79.1 ± 3.2), “relaxing” (77.4 ± 4.1), and “comforting” (77.3 ± 3.9).

Fig. 3 shows the results of the correlation coefficient test for preference for SFDJ aroma and its impressions. Regarding the relationship between preference and impressions, positive correlations were observed between preference and “elegant” (r = 0.813, p = 0.00004), “relaxing” (r = 0.786, p = 0.0001), “comforting” (r = 0.806, p = 0.00005), “refreshing” (r = 0.585, p = 0.011), “motivating” (r = 0.501, p = 0.034), and “fruity” (r = 0.475, p = 0.046).

3.3 Changes in subjective mood scale after inhalation of SFDJ aroma

The results of the comparison of mood VAS scores before and after inhalation of SFDJ aroma are shown in Fig. 4. “Subjective stress” was significantly lower after inhalation of SFDJ aroma (30.94 ± 5.59) compared with before inhalation (41.83 ± 5.41) (t (17) = 3.28, p = 0.004, r = 0.62). “Invigoration” was significantly higher
after inhalation of SFDJ aroma (55.64 ± 5.52) compared with before inhalation (40.11 ± 4.37) \( t (17) = -2.39, p = 0.028, r = 0.50 \).

### 3.4 Changes in multiple mood scale after inhalation of SFDJ aroma

The results of the comparison of MMS scores before and after inhalation of SFDJ aroma are shown in Fig. 5.
“Depression/anxiety” was significantly lower after inhalation of SFDJ aroma (7.28±0.48) compared with before inhalation (9.61±0.65) (t (17) = 4.51, p = 0.0003, r = 0.74). “Hostility” was significantly lower after inhalation of SFDJ aroma (5.28±0.23) compared with before inhalation (5.89±0.33) (t (17) = 2.83, p = 0.012, r = 0.57). “Inactivity” was significantly higher after inhalation of SFDJ aroma (15.17±0.64) compared with before inhalation (12.56±0.67) (t (17) = −3.78, p = 0.002, r = 0.68). “Affinity” was significantly higher after inhalation of SFDJ aroma (9.89±0.76) compared with before inhalation (8.94±0.76) (t (17) = −2.12, p = 0.049, r = 0.46). “Focus” was significantly lower after inhalation of SFDJ aroma (9.33±0.51) compared with before inhalation (10.33±0.70) (t (17) = 2.15, p = 0.046, r = 0.46).

3.5 Changes in pupillary light reaction after inhalation of SFDJ aroma

The results of the comparison of miosis rates after inhalation of plain hot water vapor and SFDJ aroma are shown in Fig. 6. The miosis rate was significantly higher after inhalation of SFDJ aroma (0.35±0.02) compared with after inhalation of plain hot water vapor (0.31±0.02) (t (17) = −3.56, p = 0.002, r = 0.65).

3.6 Changes in fingertip skin temperature after inhalation of SFDJ aroma

Fig. 7 shows the comparison of fingertip skin temperatures after inhalation of plain hot water vapor and SFDJ aroma. Fingertip skin temperature was significantly higher after inhalation of SFDJ aroma (31.35±1.02) compared with after inhalation of plain hot water vapor (30.4±1.04) (t (17) = −3.68, p = 0.002, r = 0.65).
3.7 Changes in brain activity after inhalation of SFDJ aroma

Table 1 shows the results of comparison of Oxy-Hb concentrations (mM/mm) in 16 channels during the last 1 minute of inhalation of plain hot water vapor and SFDJ aroma. Oxy-Hb concentrations in channel 9 (t (17) = 2.66, p = 0.017, r = 0.54), channel 10 (t (17) = 2.66, p = 0.017, r = 0.54), and channel 12 (t (17) = 2.43, p = 0.026, r = 0.51) were significantly lower when the SFDJ aroma was inhaled than when plain hot water vapor was inhaled. The Oxy-Hb concentration in channel 15 was lower (t (17) = 1.90, p = 0.075, r = 0.42) when the SFDJ aroma was inhaled than when plain hot water vapor was inhaled, but the difference was not statistically significant.

Fig. 8 shows an image of averaged Oxy-Hb concentrations in the prefrontal area of the brain 1 minute after inhalation of plain hot water vapor and SFDJ aroma. Decreases in cerebral blood flow were observed in a wide range of regions in the prefrontal area of the brain, mainly in channel 9 in the lower middle part and channels 10, 12, and 15 in the lower middle and left part, when the black tea aroma was inhaled than when plain hot water vapor was inhaled.

3.9 Association of SFDJ aroma on autonomic nervous system activity and psychological factors

The effects of SFDJ aroma on autonomic nervous system activity, preference, and psychological factors were examined. As shown in Fig. 9, there were no cor-

| Channel no. | Hot water mean ± SEM | SFDJ mean ± SEM | p   | r   |
|-------------|----------------------|----------------|-----|-----|
| 1           | 0.013 ± 0.010        | 0.047 ± 0.029  | 0.221 | 0.29 |
| 2           | -0.010 ± 0.009       | -0.007 ± 0.018 | 0.844 | 0.05 |
| 3           | -0.014 ± 0.009       | -0.026 ± 0.030 | 0.714 | 0.09 |
| 4           | -0.024 ± 0.014       | -0.053 ± 0.031 | 0.393 | 0.21 |
| 5           | -0.002 ± 0.015       | -0.008 ± 0.026 | 0.774 | 0.07 |
| 6           | -0.012 ± 0.009       | -0.044 ± 0.030 | 0.296 | 0.25 |
| 7           | -0.009 ± 0.010       | -0.031 ± 0.018 | 0.267 | 0.27 |
| 8           | -0.015 ± 0.008       | -0.048 ± 0.023 | 0.101 | 0.39 |
| 9           | -0.033 ± 0.010       | -0.107 ± 0.029 | 0.017 | 0.54 |
| 10          | -0.016 ± 0.009       | -0.073 ± 0.021 | 0.017 | 0.54 |
| 11          | -0.003 ± 0.010       | -0.026 ± 0.025 | 0.290 | 0.26 |
| 12          | -0.015 ± 0.010       | -0.079 ± 0.025 | 0.026 | 0.51 |
| 13          | -0.022 ± 0.016       | -0.042 ± 0.030 | 0.463 | 0.18 |
| 14          | -0.039 ± 0.033       | -0.045 ± 0.030 | 0.830 | 0.05 |
| 15          | -0.009 ± 0.010       | -0.050 ± 0.025 | 0.075 | 0.42 |
| 16          | -0.010 ± 0.027       | -0.002 ± 0.031 | 0.786 | 0.07 |

Each value represents mean ± SEM (n = 18). p: probability of significance, r: effect size (Paired t-test).
relations among the effects of SFDJ aroma on autonomic nervous system activity (Δmiosis rate, Δfingertip skin temperature) and psychological factors (preference for the aroma, Δdepression/anxiety, Δsubjective stress).

4. Discussion

In the present study, we performed psychological and integrative physiological evaluations to determine the effects of SFDJ aroma on psychological and autonomic and central nervous system activities in a resting state. Since the psychological and physiological effects of aromas are believed to be dependent on the sensitivity and preference to aromas9-11, we examined how the preference for SFDJ aroma is associated with its psychological and physiological effects.

Our experiment on the preference for SFDJ aroma and its impressions showed that SFDJ aroma was associated with high preference and positive impressions of “elegant,” “relaxing,” and “comforting.” Furthermore, a significant correlation was found between these impressions and the preference for SFDJ aroma, suggesting that recognition of SFDJ aroma as an elegant, relaxing, or comforting aroma may lead to high preference.

In the experiment to investigate the psychological effects of SFDJ aroma, decreases in negative emotions, namely “subjective stress”, on the mood VAS score and “depression/anxiety” and “hostility” on the MMS score, were observed, while positive emotions increased, as was evident in “invigoration” on the mood VAS score and “inactivity” and “affinity” on the MMS score. In a previous study using the same psychological questionnaire, the presentation of a citrus aroma component decreased “general fatigue” and increased “invigoration” and “clear headedness,” suggesting that the stimulation of olfactory nerves by the aroma component led to subjective improvement in fatigue and mood change26. Our study suggests that smelling the aroma of SFDJ reduces negative emotions, such as
stress, and enhances positive emotions such as invigoration and peace of mind. Notably, the changes in opposing emotions, such as a decrease in negative emotions and an increase in positive emotions, were observed simultaneously.

We evaluated the effects of SFDJ aroma on autonomic nervous system activity by measuring the pupillary light reaction, which reflects parasympathetic nerve activity, and fingertip skin temperature, which reflects sympathetic nerve activity. Unlike heart rate variability analysis, which requires respiratory control for aroma evaluation, the evaluation methods we employed have the advantage of being simple and sensitive enough to evaluate autonomic nervous system activity in a resting state under natural respiration.\(^{29-31}\) Investigation of the effects on parasympathetic nerve activity, as measured by pupillary light reaction, showed a significant increase in the miosis rate after inhalation of SFDJ aroma compared with after inhalation of plain hot water vapor. Miosis and mydriasis are governed by autonomic nervous system activity, and they stem from the reaction of the sympathetically innervated dilator pupillae and the parasympathetically innervated sphincter pupillae. When parasympathetic activity is dominant, the sphincter pupillae become more contracted and so does the pupil (the rate of contraction relative to baseline, i.e., miosis rate, increases\(^ {27,28} \)), suggesting that inhalation of plain hot water vapor and inhalation of SFDJ aroma both result in a state of parasympathetic nerve activity dominance. Previous studies have reported that warm vapors generated from plain hot water render parasympathetic nerve activity dominant\(^ {29,30} \). We demonstrated that the miosis rate was significantly higher after inhalation of SFDJ aroma compared with that of hot water vapor. Based on previous evidence, this result strongly suggests that the aroma of SFDJ itself can enhance parasympathetic nervous system activity.

Our investigation of the effect on the sympathetic nerve activity based on the measurement of fingertip skin temperature revealed a significant increase in fingertip skin temperature after inhalation of SFDJ aroma compared with plain hot water vapor. The dilation and constriction of skin peripheral vascular network are controlled by sympathetic nerve activity. Accordingly, when sympathetic nerve activity is inhibited by a certain stimulus, the peripheral vascular network dilates and blood flow increases, which elevates the skin temperature. On the other hand, when sympathetic nerve activity is increased, as in stress-loaded conditions, the peripheral vascular network contracts, and skin temperature decreases as blood flow decreases.\(^ {30} \) It is therefore conceivable that the increase in peripheral skin temperature observed after inhalation of the SFDJ aroma is ascribable to the suppression of sympathetic nerve activity and a resultant increase in skin blood flow. As previously reported in the literature, our observation of the increase in miosis rate and peripheral skin temperature after inhalation of SFDJ aroma was comparable to that of Damask rose\(^ {36} \) and Maillard reaction-derived aroma\(^ {32} \). These results suggest that smelling SFDJ aroma suppresses sympathetic nerve activity, resulting in a relative increase in parasympathetic nerve activity.

In the analysis of the effect of SFDJ aroma on the central nervous system activity, changes in Oxy-Hb concentration in the prefrontal area were measured by NIRS, which reflects brain activity. NIRS enables us to noninvasively observe the dynamics of blood volume and oxygenation in tissues at a depth of about 2 to 3 cm from the skin by utilizing the biological sensitivity of near-infrared light and the absorption spectrum changes resulting from the oxygenation and deoxygenation of hemoglobin in the blood.\(^ {33} \) When brain activity is high, oxygen consumption increases with an increase in cerebral blood flow. When brain activity is low, in contrast, oxygen consumption decreases with a decrease in cerebral blood flow.\(^ {34} \) NIRS measurement showed that the concentration of Oxy-Hb in channels 9, 10, and 12 was significantly lower when SFDJ aroma was inhaled than when plain hot water vapor was inhaled. The channels with decreased Oxy-Hb concentrations were located in the lower middle to lower left of the prefrontal area. The medial fronto-orbital area, which is located in the lower central part of the prefrontal cortex, integrates sensory information and is related to expression, decision-making, and expectation. It is also said to control reward-related motion planning, playing an important role in emotion and motivation.\(^ {34,35} \) A previous study of blood flow changes in the medial fronto-orbital area has reported that an arousing aroma component adds to blood flow mainly in the medial orbital area and that a sedating aroma component decreases blood flow in the medial orbital area.\(^ {36} \).
The decrease in cerebral blood flow induced by inhalation of the SFDJ aroma was comparable to that of the already reported Damask rose aroma and Maillard reaction-derived aroma. It has also been reported that pleasant odors are perceived in the left hemisphere of the brain, while unpleasant odors are perceived in the right hemisphere, and that pleasant odors alter the activity pattern of the prefrontal cortex from right to left dominance in response to mental stress. Based on these preceding studies, the reduction of blood flow from the lower middle to the lower left part of the prefrontal area observed during the inhalation of black tea aroma in the present study may have been associated with the perception of black tea aroma as a pleasant aroma, leading to a sedative effect on central nervous system activity.

It has been reported that essential oils, such as bergamot and neroli, have a sedative effect while lemon and orange have an arousing effect, and that blends of several essential oils are more effective than single essential oils as the blends are generally perceived as more pleasant. Since the human brain tends to reject unpleasant aromas, the pleasantness and unpleasantness of an aroma likely impacts its overall effect. In a previous study, a psychological and physiological sedative effect was observed in a group with high preference for the aroma of jasmine tea, while no sedative effect was observed in a group with low preference because the aroma of jasmine tea acted as a stressor. In addition, studies suggest that the physiological effects of aromas depend on personal preference and their psychological effects; for example, subjective unpleasantness of smelling a bad odor triggers a stress response related to sympathetic nerve activity.

We examined the association between the physiological and psychological effects of SFDJ aroma on autonomic nervous system activity and demonstrated that the sedative effects shown by the increase in miosis rate and peripheral skin temperature were not correlated with the high preference for the aroma, reduction of "subjective stress", nor reduction of "depression and anxiety". The results suggest that the psychological effects of SFDJ aroma, such as the pleasantness and displeasure of the aroma, are not the only factors that contribute to its sedative effects on the autonomic nervous system activity. In fact, studies suggest that the psychological effects do not necessarily have an influence on physiological effects. Recently, Ohata et al. investigated the psychological and physiological effects of 2,3-dimethylpyrazine and 2,5-dimethyl-4-hydroxy-3(2)-furanone, which are the aroma components produced during the Maillard reaction, and they found that 2,3-dimethylpyrazine, which has a faint scent with a slight psychological effect, produces a higher physiological effect even at a lower concentration than 2,5-dimethyl-4-hydroxy-3(2)-furanone, which is highly aromatic and psychologically effective. A study examined the effect of cedrol, an aroma component isolated from coniferous forests, such as the Himalayan cedar, and demonstrated that it had physiological sedative effects in 30% of all subjects, even at concentrations in which the aroma was completely imperceptible. Furthermore, an analysis of the pathway of action of Cedrol suggested that in addition to the upper respiratory tract with known olfactory pathways, the lower respiratory tract, from the vagus nerve in the pharynx to the central nervous system, was also involved in processing cedrol. In addition to the olfactory pathway, aroma is also processed via the transdermal and intranasal absorption pathways. For example, linalool is a sedative aroma compound known to enter the brain via the bloodstream. Similarly to 2,3-dimethylpyrazine and Cedrol, the physiological sedative effects of SFDJ aroma are independent of its psychological effects. Since Linalool is also a major component of black tea, it is possible that one of the aroma components in SFDJ may have exerted its effect through the bloodstream, acting directly on the brain. However, it is difficult to elucidate the correlation between the psychophysiological responses to SFDJ aroma because the relationship between the aroma components involved in the effects and the action pathways is unclear given the present evidence.

Aroma exposure triggers a stress response in the brain, and any aroma is known to elicit either a pleasant stress, in which the aroma has a positive effect, or an unpleasant stress, in which the aroma has a negative effect. In the present study, SFDJ aroma had psychophysiological sedative effects even under non-stress conditions, suggesting that it is a pleasant stress aroma. We also demonstrated that the preference for SFDJ scent was higher than that for AS, UV, and EG aromas. Preliminary studies also suggest that SFDJ
aroma has high psychophysiological effects (results not shown). While our findings indicated the involvement of components unique to SFDJ aroma, it is necessary to consider various stress situations and to search for the components involved in order to fully elucidate the factors causing the sedative effects. To date, more than 600 aroma compounds have been identified in black tea\(^\text{13}\). These include Linalool\(^\text{48}\) and Geraniol\(^\text{49}\), which have already been reported to be effective. Thus, it is not difficult to imagine that one of the aroma components is involved in the psychophysiological effects of SFDJ aroma. In future research, it is necessary to clarify the aroma components of SFDJ and to analyze the concentration dependence of the psychophysiological and physiological effects of these aroma components, as well as to study in detail the factors that cause the sedative effects of SFDJ aroma.

5. Conclusions

Our findings indicate that individuals tend to have high preference for SFDJ aroma, and that the aroma had a sedative effect against psychological and physiological stress. Inhalation of black tea aroma led to psychological changes characterized by a decrease in negative emotions such as "subjective stress," "depression/anxiety," and "hostility," and an increase in positive emotions such as "invigoration," "inactivity" and "affinity".

In terms of autonomic nervous system activity, we observed an increase in miosis rates and fingertip skin temperature. This indicated that the sympathetic nerve activity was suppressed and parasympathetic nerve activity was elevated. We also observed a significant decrease in blood flow in the prefrontal area of the brain, which indicated that the black tea aroma had a sedative effect on central nervous system activity. The physiological sedative effect of SFDJ on autonomic nervous system activity was not correlated with the level of preference or psychological effects. Therefore, our findings suggest that the physiological sedative effects of SFDJ aroma are independent of psychological effects such as liking or disliking of the scent itself and pleasure or discomfort.

**Key words**: second flush Darjeeling tea aroma, preference, autonomic nervous system activity, central nervous system activity, psychology, sedative effect

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ダージリン紅茶セカンドフラッシュの香りが
心理と自律神経活動・中枢神経活動に及ぼす効果

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要旨: 本研究の目的は、ダージリン紅茶セカンドフラッシュの香りによる心理生理的な作用を明らかにする
ことである。ダージリン紅茶セカンドフラッシュの香りに対する嗜好性と香りの印象を評価したうえで、心
理学的評価とあわせて、自律神経および中枢神経活動の統合生理学的評価を行った。その結果、ダージリン
紅茶セカンドフラッシュの香りを吸入すると“自発的ストレス”、“抑制・不安”“敵意”が有意に低下し
た。一方、”爽快感”“非活動的”は有意に上昇したことから、心理に対して鎮静的に作用することが示唆
された。生理的には、ダージリン紅茶セカンドフラッシュの香り吸入後に瞳孔の縮瞳率と末梢皮膚温が有意
に上昇したことから、交感神経活動が抑制され、副交感神経活動が優位な状態になることが示唆された。さ
らに、脳の前頭前野部で有意な血流低下が観察されたことから、中枢神経活動に対して鎮静的に作用するこ
とが示唆された。ダージリン紅茶セカンドフラッシュの香りにより心理生理的な鎮静作用が認められたが、自律
神経活動に対する生理作用と、香りの嗜好性および心理作用との間に相関関係は認められなかった。

キーワード: ダージリン紅茶セカンドフラッシュの香り、自律神経活動、中枢神経活動、心理、鎮静作用、
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