Inhalation of Japanese cedar (Cryptomeria japonica) wood odor causes psychological relaxation after monotonous work among female participants

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ABSTRACT
Essential oils have potential to mitigate stress symptoms and treat symptoms related to mental health. Few studies have investigated the effects of wood-derived aromatics on endocrinological and psychological responses in an actual space. In this study, we evaluated the effects of essential oil derived from Japanese cedar (Cryptomeria japonica) wood on the recovery state of female participants after they performed monotonous work. We determined the levels of salivary stress markers to describe the endocrinological responses. And we also used questionnaires to assess the perception of the odor of experimental rooms and psychological states. We found that olfactory stimulation with the volatile compounds of essential oil derived from Japanese cedar wood modulates mood states, and may transiently decrease sympathetic nervous activity. We suggest that olfactory stimulation with the volatile compounds of essential oil derived from Japanese cedar wood could be useful for maintaining mental health among women.

Essential oil is an aromatic plant constituent. In addition to imparting characteristic flavors and odors to certain plants, essential oils are partly responsible for the pharmaceutical properties of aromatic herbs. Essential oils could mitigate stress symptoms, for example, elevated blood pressure and heart rate (10, 11, 13), and effectively treat symptoms related to mental health, such as mood swings and depression (8, 18, 34). Olfactory stimulation with essential oils has potential pharmaceutical and therapeutic benefits for stress management. A brief break with olfactory stimulation with aromatic components may ameliorate fatigue caused by continuous work (1).

Worldwide, wood is used as a structural material given its ubiquity. Volatile compounds derived from wood have pharmacological effects (4, 15, 19), and information on the effects of olfactory stimulation with wood-derived volatiles on the human body has considerably accumulated. The individual volatile compounds emitted from wood chips and essential oil affect the human autonomic nervous system (5, 14, 32). However, few studies have investigated the effects of wood-derived aromatics on endocrinological and psychological responses during recovery after stressful work in an actual space, especially in women.

For many workers, complex workplace factors, such as heavy workloads could promote mental health disturbances (6, 21, 33). And women experience a specific physiological cycle that strongly influences the secretion of neurological and physiological hormones. The range of emotional fluctuation in women is more drastic than that in men; and the incidence rate of depression, a major mental disease, is higher in women than in men (23).

We tried to evaluate physiological and psychological relaxation effects of essential oil derived from Japanese cedar (Cryptomeria japonica) wood with female participants. In Japan, Japanese cedar is the most commonly planted tree in forests, and its timber is often used as an interior decorating material. In the present study, we used a calculation task to
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simulate monotonous work. We determined the levels of salivary stress markers to describe the endocrinological responses of the participants before and after the task. We also used questionnaires to assess the perception of the odor of experimental rooms and psychological states.

MATERIAL AND METHODS

Participants and experimental procedure. The experimental procedure of the study was approved by the Forestry and Forest Products Research Institute and was in accordance with the Declaration of Helsinki. Twenty-nine participants (female, age: 21.6 ± 1.7 years (mean ± SD); range: 20–29 years) were recruited. None of the participants had physical or mental health abnormalities, and none were using prescription drugs or smoking at the time of the study. Women undergoing menstruation were excluded. The purpose and schedule of the experiments were explained. Written informed consent was obtained from all participants prior to study initiation. Caffeine consumption on the day of the experiment was prohibited.

An experimental room in our research institute (17) was used. The specification of the experimental room is shown in Fig. 1, and the experimental procedure is shown in Fig. 2. Each participant performed the experiment once in the absence (control treatment) or in the presence (wood treatment) of essential oil derived from Japanese cedar.

We used Japanese cedar from Kitayama (Kyoto, Japan) as an experimental material and obtained essential oil (17). To measure psychophysiological effects, we filled a diffuser (Personal Diffuser Squair; AT-AROMA Co. Ltd., Tokyo, Japan) with a dilute solution containing Japanese cedar essential oil. For the wood condition, 0.4 mL of the essential oil was mixed with 3.6 mL of a dilute solution according to the diffuser instruction manual for diffusion into the experiment room. Another 4.0 mL of the dilute solution only was prepared for diffusion for the control condition.

Simulation of monotonous work with arithmetic work. We used the Uchida–Kraepelin test (Nisseiken Inc., Tokyo, Japan), which is a serial addition test that requires takers to perform calculations as quickly and accurately as possible. Each participant was supplied with a preprinted sheet of paper containing 15 lines of random, single-digit, horizontally aligned numbers. The participant was instructed to calculate the numbers of a specified line and to move to a new line every minute. This test was conducted for 30 min.

Subjective assessments. We used five questionnaires for subjective evaluation. Three questionnaires for the subjective assessment of the odor of experimental rooms were described in the previous study: irritation scale and hedonic scale, a visual analog scale (VAS) (17). And subjective fatigue was also measured with VAS: “does not feel tired at all–completely exhausted.” Regarding the VAS scale, participants were asked to mark the assessment along a continuous line between each of the two end-points. The Japanese version of the Profile of Mood States Short Form (POMS-SF) (Kaneko Shobo Co., Tokyo, Japan) was administered to assess mood states. Each item was rated on a 5-point Likert-type scale of 0–4 ranging from “not at all” to “extremely.” Raw scores were added to generate six subscales of emotional state: tension–anxiety (T–A), depression–dejection (D), anger–hostility (A–H), vigor (V), fatigue (F), and...
Relaxation by Japanese cedar odor

We analyzed the volatile compounds of Japanese cedar wood through GC-MS. Similar to those found in our previous study, the primary constituents of the oil included δ-cadinene, 4-epi-calamin, and confusion (C). These added raw scores were converted into T-scores in accordance with the POMS manual (37). The irritation scale and hedonic scale questionnaires were administered at 0 and 30 min after work that was during the rest period. The VAS questionnaire of room odor was performed at 0 min of the rest period. Subjective fatigue was assessed at prework and at 0 and 30 min of the rest period. POMS was performed at prework and at 30 min of the rest period. Emotional and subjective responses were assessed at prework and at 0 and 30 min of the rest period.

Salivary stress marker assay. We used a cotton swab (Salivette; Sarstedt AG & Co., Nürnberg, Germany) to collect saliva samples for the measurement of stress markers. Each participant swabbed her mouth for 1 min. The swab was kept on ice. Saliva was collected through centrifugation at 1500 × g for 15 min and kept at −20°C until use. Saliva was collected at prework and four times during the rest period. Salivary cortisol and dehydroepiandrosterone sulfate (DHEA-s) were measured with an enzyme immunoassay kit (Salimetrics, PA, USA). Chromogranin A (CgA) was measured with an enzyme immunoassay kit (Yanaihara Industries Inc., Shizuoka, Japan). Salivary α-amylase was measured using a salivary amylase monitor (Nipro Co., Osaka, Japan). The meter tip was immersed in saliva under the tongue of the participant for 30 s and measured within 2 min after collection.

Gas chromatography-mass spectrometry analysis. We used PEJ-02 tubes (Sigma-Aldrich, MO, USA) to collect volatile organic compounds from the experimental room treated with essential oil. The analysis methods of volatiles with an automatic thermal desorption system and a gas chromatography-mass spectrometry (GC-MS) system were described in the previous study (17). We compared the collected GC-MS data with those retrieved from a mass spectral database library (NIST14) and calculated the concentrations of the target compounds in the sample using the calibration standard line of β-caryophyllene (Sigma-Aldrich).

Statistical analysis. All values are expressed as mean ± S.E.M. The values of salivary parameters were calculated on the basis of prework values. Two-way ANOVA and Student’s t-test were conducted to compare differences in salivary parameters between experimental conditions and one-way ANOVA with repeated measures and posthoc comparisons with Bonferroni were performed for each condition. The Wilcoxon signed-rank test and Mann–Whitney U test were performed to compare within- and between-condition differences in the subjective assessment of the irritation and hedonic scales and POMS, respectively. Friedman test was performed to compare subjective fatigue and subjective responses between conditions. Statistical significance was recognized when the P value was <0.01 or <0.05. All statistical analyses were performed using SPSS 25.0J for Windows (SPSS Japan, Tokyo, Japan).

RESULTS
Constituent analysis of essential oil from Japanese cedar wood
We analyzed the volatile compounds of Japanese cedar wood essential oil through GC-MS. Similar to those found in our previous study, the primary constituents of the oil included δ-cadinene, 4-epi-calamin.
cubebol, cubebol, and several sesquiterpenes. The total volume of the volatile compounds of Japanese cedar wood dissipated in the experimental room was 304.6 μg/m³ and was not detected under the control condition.

**Analysis of salivary stress markers**

Salivary stress markers were measured before and after work, as described in Fig. 2. Each marker was measured at prework and at 0, 10, 20, and 30 min after work. The values for each participant during the rest period are shown as the rate of change in the prework value. The changes in cortisol levels from the prework period to the rest period are shown in Fig. 3A (experimental conditions effects: F[1, 23] = 0.37; time effect: F[4, 92] = 19.65, P < 0.01; experimental conditions × time effect: F[4, 92] = 0.24). The values in the control condition had a significant time effect ([F(4, 32) = 9.39], P < 0.01). And the values at 0 min (< 0.01) and 10 min (< 0.01), 20 min (< 0.01) of the rest period were significantly different compared with the prework values. Cortisol levels of the rest period decreased under both conditions.

The changes in DHEA-s levels from the prework period to the rest period are shown in Fig. 3B (experimental conditions effects: F[1, 25] = 0.01; time effect: F[4, 100] = 7.08, P < 0.01; experimental conditions × time effect: F[4, 100] = 11.71, P < 0.01). The values in the control condition had a significant time effect ([F(4, 60) = 11.71], P < 0.01). And the values at 0 min (< 0.01) and 10 min (< 0.01), 20 min (< 0.01) of the rest period were significantly different compared with the prework values. The values at 0 and 10 min during the rest period under the control condition and those at 0 min during the rest period in the wood condition are significantly different. (B) Bars represent the values of the changes in salivary DHEA-s levels under the control and wood conditions. The values at 0 min during the rest period under the control condition and those at 0 min during the rest period under the wood condition are significantly different. (C) Bars represent changes in salivary α-amylase levels under the control and wood conditions. Values at 20 min during the rest period under the control condition and those at 10 min during the rest period under the wood condition are not significantly different. **Statistical significance (P < 0.01), *Statistical significance (P < 0.05). Data are shown as mean ± SEM. CNT: control condition, WOOD: wood condition, PRE: before performing arithmetic work, R0: 0 min of the rest period, R10: 10 min of the rest period, R20: 20 min of the rest period, R30: 30 min of the rest period.

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**Fig. 3** Variation in salivary biomarkers at prework and during the rest period. (A) Bars represent the values of the changes in salivary cortisol levels under the control (white) and wood (black) conditions. Values during the rest period are shown as the rate of changes of prework values. The values at 0 and 10 min during the rest period under the control condition and the values at 0, 10, and 20 min during the rest period under the wood condition are significantly different. (B) Bars represent the values of the changes in salivary DHEA-s levels under the control and wood conditions. The values at 0 min during the rest period under the control condition and those at 0 min during the rest period in the wood condition are significantly different. (C) Bars represent changes in salivary α-amylase levels under the control and wood conditions. Values at 20 min during the rest period under the control condition and those at 10 min during the rest period under the wood condition are significantly different. (D) Bars represent changes in salivary CgA levels under the control and wood conditions. Values under control and wood conditions are not significantly different. **Statistical significance (P < 0.01), *Statistical significance (P < 0.05). Data are shown as mean ± SEM. CNT: control condition, WOOD: wood condition, PRE: before performing arithmetic work, R0: 0 min of the rest period, R10: 10 min of the rest period, R20: 20 min of the rest period, R30: 30 min of the rest period.
The changes in α-amylase levels from the prework to the rest period are shown in Fig. 3C (experimental conditions effect: F[1, 20] = 1.65; time effect: F[4, 80] = 7.56, P < 0.01; experimental conditions × time effect: F[4, 80] = 0.84). The values in the control condition had a significant time effect (F[4, 32] = 3.60, P < 0.05). And the values at 0 min (P < 0.01) of the rest period were significantly different compared with the prework values. The values in the wood condition had a significant time effect (F[4, 64] = 4.23, P < 0.01). And the values at 0 min (P < 0.01) of the rest period were significantly different compared with the prework values. DHEA-s levels at 0 min of the rest period were higher than those at different time points under both conditions.

The changes in α-amylase levels from the prework period to the rest period are shown in Fig. 3D. The values in the wood condition especially at 0 min of the rest period were significantly different compared with the prework values. The values in the control condition had a significant time effect (F[4, 36] = 3.26, P < 0.05). And the values at 0 min (P < 0.05) of the rest period were significantly different compared with the prework values. The changes in irritation were significantly different (P < 0.01). Anger–hostility (A–H), vigor (V), and confusion (C) under the wood and control conditions were not significantly different (Fig. 6).

DISCUSSION

In the present study, we found that olfactory stimulation with volatile compounds derived from Japanese cedar wood may potentially induce psychologically refreshing effects after performing stressful work. Providing workers with relaxation spaces suffused with volatiles derived from the essential oil of Japanese cedar wood may help prevent mental health disturbances. The present study indicated that the utilization of wood essential oils for mental health may expand the market for aromatic products and may improve the office environment.

Consistent with those identified in previous reports, we identified that the volatile compounds emitted from Japanese cedar included δ-cadinene, 4-epi-cubeol, cubebol, and other sesquiterpenes (3, 22). The total volatile concentration was 304.6 (μg/m³). Regarding dilution rate of essential oil, we ensured that all volunteers felt comfortable and did not experience dysfunction e.g., headache and coughing in the preliminary test. Previous studies suggested that the total volume and types of volatiles are important factors of a healthy indoor environment (2, 35). We also suggest that these factors provide a comfortable, relaxing, and safe workplace. Future studies should determine the optimal concentration of essential oil from Japanese cedar wood and the contribution of each volatile compound to psychophysiological effects.

The measurement of biomarkers in saliva is a
Changes in salivary biomarkers, cortisol, CgA (29, 30), α-amylase levels (38) and DHEA levels (9) under olfactory stimulation have been reported in male and female participants and indicated relieving stress. In this study, we focused on the time-dependent changes in endocrinological and sympathetic nervous activities in the participants. We selected salivary cortisol and DHEA-s levels as endocrinological parameters. Cortisol is an adrenocortical hormone and is crucial for the regulation of the homeostasis of the internal body environment (24, 29, 31). DHEA-s is a sulfated metabolite of DHEA, which is an androgen precursor secreted by the adrenal cortex in the hypothalamic–pituitary–adrenal axis. An earlier study demonstrated that the secretion of DHEA-s and the response of the hypothalamic–pituitary–adrenal axis are related; moreover, DHEA-s has the potential to be used as a biomarker for stress (26). α-Amylase and CgA have

**Fig. 4** Subjective assessments of odor of the experiment rooms. (A) Bars represent irritation scores obtained under control (white) and wood (black) conditions. The differences due to time are remarkable \( (P < 0.01) \) under both experimental conditions but were not significant at different sampling times. (B) Bars represent hedonic scores under control and wood conditions. No significant differences exist between conditions. Data are shown as mean ± SEM. (C) Bars represent the visual analog scale (VAS) scores under control and wood conditions. VAS scores are not significantly different between conditions. **Statistical significance \( (P < 0.01) \), *Statistical significance \( (P < 0.05) \). Data are shown as mean ± SEM. CNT: control condition, WOOD: wood condition, R0: 0 min of the rest period, R30: 30 min of the rest period.

**Fig. 5** Subjective assessments of psychological fatigue. Bars represent subjective fatigue scores obtained under the control (white) and wood (black) conditions. Differences due to time schedule are remarkable under both experimental conditions. Assessments are not significantly different at different sampling times under both conditions. **Statistical significance \( (P < 0.01) \). Data are shown as mean ± SEM. CNT: control condition, WOOD: wood condition, PRE: before performing arithmetic work, R0: 0 min of the rest period, R30: 30 min of the rest period.
Relaxation by Japanese cedar odor

The changes in both parameters showed that physiological relaxation was induced when the hyperactivities of the sympathetic nervous system were suppressed through the inhalation of volatiles emitted from Japanese cedar wood. Specifically, CgA and α-amylase levels decreased during the rest period. A previous study showed that volatiles emitted from Japanese cedar wood suppress increases in CgA and α-amylase levels (16). Several studies have also indicated that transient olfactory stimulation with volatiles derived from Japanese cedar wood decreases sympathetic nervous activity regardless of gender (14, 32).

Five questionnaires were administered for the subjective assessments of sensitivity and psychological feelings. Sensitivity evaluation showed that changes in irritation significantly differed with time under each condition, whereas hedonic responses did not significantly differ. VAS scores for the odor of the experimental rooms under the wood and control conditions did not significantly differ (Fig. 4A and 4B, 4C). In this study, we set the concentration of essential oil from Japanese cedar wood in the experimental room to comfortable and/or weak odor on the basis of our preliminary evaluation. We suggest that the level of essential oil may have contributed to the negligible differences in sensitivity evaluation.

The POMS test better revealed the changes in psychological feelings, subjective fatigue, emotional levels, and perception of experimental rooms than the self-made VAS scale (Figs. 5 and 6). T–A, D, and F scores significantly decreased only under the wood condition (Fig. 6). These results indicated that olfactory stimulation with volatiles derived from Japanese cedar wood reduces emotional anxiety, de-

been used as markers of the activation of the sympathetic nervous system under stress. Salivary α-amylase is an enzyme released by the salivary glands under the control of the sympathetic nervous system (7, 27). CgA is an acidic glycoprotein produced by the submandibular glands; it is secreted into the saliva and released with catecholamine from sympathetic nerve endings (20, 25). Our results indicated that experimental time has a significant effect on the levels of α-amylase, cortisol, and DHEA-s (Fig. 3A and 3B, 3C). However, the interaction between experimental time and conditions did not significantly change in all biomarker levels. We previously found that DHEA-s levels increase in response to the inhalation of volatiles derived from Japanese cedar wood (17). The difference between the results of our previous and present studies may be attributed to physiological differences among the participants in the present study. Although women undergoing menstruation were excluded from the present study, the participants may be in their follicular development, ovulation, or luteal phases during the time of the experiment.

The time-dependent changes in α-amylase and CgA levels observed in this study were notable (Fig. 3C and 3D). In this study, the participants entered the experimental rooms immediately after performing stressful arithmetic work. We focused on the changes in α-amylase and CgA levels during this period (R0). The changes in preexperimental (PRE) and postexperimental (R0) α-amylase and CgA levels were calculated and statistically analyzed through Student’s t-test. The results indicated that α-amylase ($P = 0.113$) and CgA ($P = 0.035$) levels significantly differed between experimental conditions. The changes in both parameters showed that physiological relaxation was induced when the hyperactivities of the sympathetic nervous system were suppressed through the inhalation of volatiles emitted from Japanese cedar wood. Specifically, CgA and α-amylase levels decreased during the rest period. A previous study showed that volatiles emitted from Japanese cedar wood suppress increases in CgA and α-amylase levels (16). Several studies have also indicated that transient olfactory stimulation with volatiles derived from Japanese cedar wood decreases sympathetic nervous activity regardless of gender (14, 32).

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Fig. 6 Subjective assessments of mood states. Bars represent POMS (Profile of Mood States) scores obtained under the control (white) and wood (black) conditions. T–A (tension–anxiety), D (depression–dejection), and F (fatigue) parameters are significantly different at different time points under control and wood conditions. A–H (Anger–hostility), V (Vigor), C (Confusion). *Statistical significance ($P < 0.05$). Data are shown as mean ± SEM. CNT: control condition, WOOD: wood condition, PRE: before performing arithmetic work, R30: 30 min of the rest period.
pression, and fatigue. Negative emotions, like anxiety, depression, and fatigue, are related to mental dysfunction. Our results suggested that olfactory stimulation with the wood-derived volatiles reduces negative feelings. Previous studies indicated that olfactory stimulation with yuzu (Citrus junos Sieb) fragrance decreases T–A, D, A–H, and C scores (18). Moreover, the scores of negative emotional parameters decrease in response to olfactory stimulation with Cyperi rhizoma essential oil (38). F scores decrease and V scores increase in response to the inhalation of lemon fragrances (12). These materials and our material contain different volatile compounds, and we obtained weaker responses than those obtained by previous studies. In future studies, we will consider the contributions of volatile compounds to emotional states and their relationships with physiological responses in detail.

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