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VOCs emitted from Japanese cedar (*Cryptomeria japonica*) interior walls induce physiological relaxation

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ABSTRACT

Wood has been used since ancient times as a building material around the world. One of the characteristics of wood is volatile organic compounds (VOCs), which indicates the relaxant effects on human body. In this study, we attempt to evaluate the psychophysiological responses to VOCs emitted from interior walls containing Japanese cedar (*Cryptomeria japonica*). Japanese cedar is the most commonly planted in forests and the timber has often been used as an interior material in Japan. The evaluation indices of the participants are arithmetic performance, subjective assessments of VOC, salivary stress markers, and temporal changes in heart rate and autonomic nerve activity. We found that the inhalation of air containing VOCs emitted from Japanese cedar interior walls suppresses the increase in salivary α-amylase activity and chromogranin A secretion. And it causes participants to feel that the odor is pleasant. The present study thus indicated that VOCs emitted from Japanese cedar walls affect the autonomic nervous system and emotion. Therefore, we suggested that an interior containing Japanese cedar can help people maintain an optimum living environment.

1. Introduction

Studies about the habitability of rooms with wooden walls have been regarded as important. Interior wood materials physiologically relax the human body through the visual and tactile pathways [1–4]. Knowledge of the psychophysiological effects of inhalation of volatile organic compounds (VOCs) of wood has also grown considerably. Several previous reports [5–8] have indicated that the essential oils of wood cause the suppressive effects of sympathetic nervous activity. These effects were obtained with using an odorant delivery system controlled by a constant-flow olfactometer, and the condition was differed from that in the room with wooden walls. There has been little study on the effects of the odor in full-scaled wooden rooms on psychophysiological responses.

Several studies have suggested that olfactory stimulation could be useful to ameliorate the fatigue caused by continuous work and to improve work performance [9,10]. Matsubara et al. suggested that volatile compounds could be utilized to maintain a high level of work performance [11]. In the present study, we used Japanese cedar (*Cryptomeria japonica*) and set up a wood interior experimental room. In Japan, Japanese cedar is the most commonly planted in forests and the timber has often been used as an interior material. We used the experimental room to evaluate the effects of VOCs emitted from Japanese cedar on individuals. We used monotonous work as a stress task, salivary stress markers and electrocardiography (ECG) to evaluate physiological responses in subjects during and after the performance of the task, and a questionnaire to evaluate the subjective assessment of VOCs emitted from Japanese cedar.

2. Methods

2.1. Chemicals

β-Caryophyllene and (+)-cedrol were purchased from Sigma–Aldrich Japan Co. (Tokyo, Japan), and α-humulene and β-eudesmol were purchased from ChromaDex Inc. (Irvine, CA, USA).

2.2. Gas chromatography–mass spectrometry (GC–MS) analysis

VOCs in this room were collected with a carbon tube (ORBO91T, Sigma–Aldrich) maintained at 22.0 ± 0.7 °C by applying a flow rate of 0.1 L min^{-1} for 24 h. The VOCs were eluted by acetone and

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analyzed by a GC–MS system (GCMS-QP2010; Shimadzu Co., Ltd., Kyoto, Japan). The machine was equipped with an Ultra ALLOY-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Frontier Laboratories Ltd., Fukushima, Japan). The temperature program was as follows: 50 °C for 3 min, followed by increases of 15 °C/min^{-1}–150 °C, 4 °C/min^{-1}–170 °C, 20 °C/min^{-1}–250 °C, and holding for 5 min. The other parameters were as follows: injection temperature, 250 °C; ion source temperature, 250 °C; carrier inlet pressure, 100 kPa; He, 1.69 mL min^{-1}; injection volume, 1 μL. We compared the GC–MS data with a mass spectral database library (NIST08) and commercially available reagents for substance estimation. And we calculated the concentrations of the target compounds in the sample using the calibration standard line of each reagent. Because several compounds in the sample were not commercially available, the standard line for quantitative analysis was taken as the area of the β-caryophyllene peak and calculated by using the calibration standard line of β-caryophyllene.

2.3. Specifications of the experimental rooms

We used forty-aged Japanese cedar from Oguni (Kumamoto, Japan) as an experimental material. The material was dried at 45 °C and processed into vertical-grain timber, after which grooves were cut into them as shown in Fig. 1a [12]. Portions of two of the walls in the experimental room were covered by these timbers for a total area of 5.69 mm^2 (Fig. 1b). The construction of the room was performed at September 2012 and the room was kept without air conditioning for six weeks. The experimental room in our university (RC structure, inside dimensions: W5040 × D3270 × H2500 mm) was used. A partition was used to prevent the timber from having any visual influence on the participants. We called this experimental room the Japanese cedar condition. Other experimental room which was the same except that it had no Japanese cedar, was called the control condition. The experimental rooms were maintained at 23 ± 2 °C throughout the experiment.

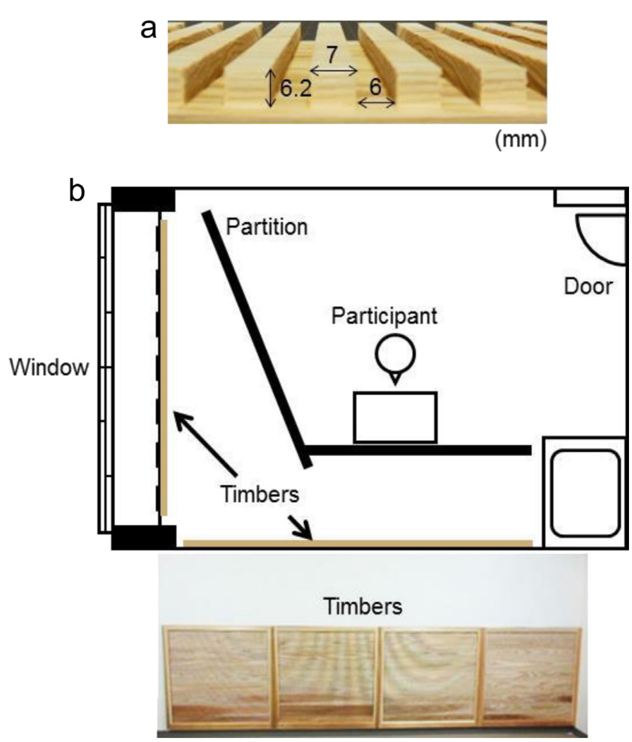

Fig. 1. a Slitted Japanese cedar timbers Grooves were cut into the timbers. The grooves were 6.2 mm deep and 6 mm wide. The vertical-grain timber between the grooves was 7 mm wide. b Specification of the experimental room with Japanese cedar The interior panels of the grooved timbers were constructed as waist-high partition walls. We used two panel sizes: V950 mm × H950 mm and V730 mm × H950 mm. In total, seven panels were used.

2.4. Participants and experimental design

The experimental design of the study was approved by Kyoto University and was in accordance with the Declaration of Helsinki. Sixteen healthy male university students (age: 23.5 ± 1.8 years (mean ± SD); range: 21–28 years) were recruited. None of the participants had any abnormality in their physical or mental health, and none were current prescription drug users or current smokers. The purpose and schedule of the experiments were explained. Written informed consent was obtained from all participants prior to study initiation. Consumption of alcohol or medications one day before the experiment was prohibited, as was consumption of caffeine on the day of the experiment. Fig. 2 shows the experimental design. Saliva was collected from all participants immediately before and after they performed the arithmetic. An electrocardiogram (ECG) recording was done during the arithmetic work. Each participant performed the experiment twice, with an interval of one week: once in the absence (control condition) and once in the presence (Japanese cedar condition) of volatiles emitted from the Japanese cedar.

2.5. Arithmetic work

We used the U–K test [13], which is a serial addition test that requires takers to perform calculations as quickly and accurately as possible. Supplied with pre-printed paper containing 15 lines of random, single-digit, horizontally aligned numbers, each participant was instructed to calculate the numbers of a specified line and to move to a new line every minute. This test was performed for repeated cycles of 15 min of work and 5 min of rest.

2.6. Subjective assessment of VOCs emitted from Japanese cedar

The subjective effects of the volatiles emitted from the Japanese cedar were measured after the arithmetic work. As a result of our preliminary screening of several words to describe the effects, eight words were selected. A visual analogue scale (VAS) was used to identify the position of each word along a continuous line between two end-points. These consisted of an eight-item questionnaire designed to differentiate subjective responses to VOCs emitted from Japanese cedar: "can't concentrate–can concentrate", "dislike–like", "feel warm–feel cold", "uncomfortable–comfortable", "feel restless–feel calm", "artificial–natural", "feel coziness–not feel coziness", and "bad odor–good odor".

2.7. ECG recording

To evaluate cardiac autonomic nervous activity, we performed a power spectral analysis of R–R intervals using a MemCalc system (MemCalc Ver. 2.5; Suwa Trust Co., Tokyo, Japan). ECG recordings were played back from a two-channel recorder, and the signals were digitized using a 12 bit analogue-to-digital converter at a sampling rate of 1 kHz. We analyzed the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) components.

2.8. Salivary stress markers assay

Salivary *a*-amylase (AA) 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

Period	Pre-work	Rest	Work	Rest	Work	Rest		Post-work
Time		5 min	15 min	5 min	15 min	5 min		
Meas.	Saliva	ECG →						Saliva

Subjective assessment

Fig. 2. Experimental procedure.

within 2 min after collection. We used a cotton swab (salivette, Sarsteds AG & Co., Nümbrecht, Germany) to measure other parameters. Each participant swabbed his mouth for 1 min, and this swab was kept on ice. Saliva was collected by centrifuge at 1500 $\times$ g for 15 min and kept at -20 °C before use. Salivary cortisol and secretory immunoglobulin A (IgA) were measured by an enzyme immunoassay (EIA) kit (Salmetrics, PA, USA). Salivary chromogranin A (CgA) was measured by an enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., CA, USA).

2.9. Statistical analysis

In all results, the values are expressed as the means $\pm$ s.e.m. To compare differences in work performance and salivary parameters, subjective assessment between the conditions, Student's *t*-test, and the Wilcoxon signed-rank test were used. For the HF and LF/HF, one-way ANOVA was used. Statistical significance was recognized when the p value was <0.05 and the tendency was $p < 0.1$. All statistical analysis was performed using SPSS 17.0J for Windows (SPSS Japan, Tokyo, Japan).

3. Results

We investigated the effects of VOCs emitted from Japanese cedar during and after continuous work. Salivary collection and ECG recordings were done in our experimental design (Fig. 2). We analyzed the VOCs emitted from Japanese cedar in the experimental room. During the work period, the participants performed the arithmetic and took the rest period while remaining seated and quiet.

3.1. Constituent analysis of the VOCs emitted from Japanese cedar

We analyzed the chemical compounds emitted from the Japanese cedar used in this study by GC–MS. The results showed that the VOCs were composed mainly of δ-cadinene, α-muurolene, α-cubebene, β-cubebene, and other several sesquiterpenes (Table 1). The total volume of VOCs in the experimental room was 498.8 (μg/m^3). VOCs were undetected in the control room.

Table 1
Chemical compounds emitted from Japanese cedar during the experiment.

RT (min)	Components	Composition (%)	RT (min)	Components	Composition (%)
5.8	α-pinene	0.6	12.4	γ-amorphene	1.5
10.7	δ-elemene	0.4	12.5	γ-muurolene	0.4
10.8	α-cubebene	4.4	12.7	C15H24	13.9
11.2	α-copaene	2.3	12.7	α-murolene	11.6
11.3	β-cubebene	3.9	12.9	Cupanene	0.8
11.5	C15H24	0.4	13.0	C15H24	14.0
11.6	C15H24	0.3	13.1	δ-cadinene	23.0
11.7	β-cedrene	2.0	13.1	C15H24	2.4
11.7	β-caryophyllene	2.8	13.2	Cadina-1, 4-diene	1.3
11.8	C15H24	0.6	13.4	α-calacorene	0.4
11.8	γ-cadinene	0.3	13.4	Elemol	0.4
11.9	Thujopsene	2.5	14.0	Gleenol	0.5
12.1	Muurola-5-diene	0.7	14.2	C15H26O	0.5
12.2	α-humulene	1.7	14.4	Cedrol	1.3
12.3	Cadina-1,4-diene	0.3	14.8	C15H26O	1.9
12.3	C15H24	0.2	15.0	C15H26O	1.2
12.4	C15H24	1.1	15.1	β-eudesmol	0.4

3.2. Arithmetic performance

Performance was determined by the total number of correct calculations made. This was defined as the average of total work time (Fig. 3). No difference in performance was found between the Japanese cedar condition and the control condition.

3.3. Subjective assessment of VOCs emitted from Japanese cedar

The level of subjective effects of Japanese cedar was determined by an eight-item questionnaire using a visual analogue scale (VAS). The VAS was a horizontal line, 100 mm in length, anchored by a word descriptor at each end. The participants marked a point on the line indicating their subjective response to each aroma. The VAS score was determined by measuring in millimeters from the center of the line to the point that marked. The VAS scores showed significant differences between the Japanese cedar condition and the control condition: scores of "bad odor–good odor" were 6.7 $\pm$ 0.4 for the Japanese cedar condition and 5.5 $\pm$ 0.2 for the control condition ($P < 0.05$) (Fig. 4).

3.4. Analysis of cardiac autonomic nervous activity during the arithmetic work

Autonomic nervous activity was analyzed by the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) components. The values of the HF components and the ratios of LF/HF

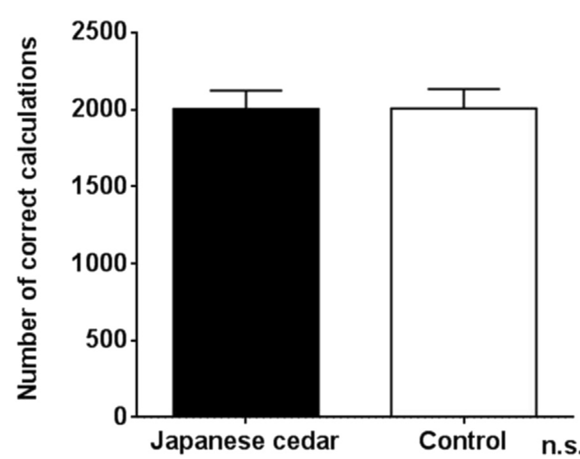

Fig. 3. Total number of correct calculations in arithmetic work. The participants were instructed to calculate the random, single-digit, horizontally aligned numbers printed on the test paper. The total number of correct calculations is shown. The bars represent the total number of correct calculations in the Japanese cedar (black) and control conditions (white). There were no significant differences. Data are shown as means $\pm$ SEM.

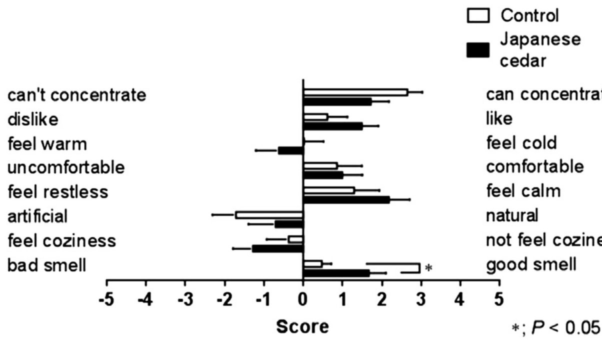

Fig. 4. Subjective effects of the volatiles emitted from Japanese cedar. The visual analogue scale (VAS) scores for the eight-item questionnaire are shown: "can't concentrate–can concentrate", "dislike–like", "feel warm–feel cold", "uncomfortable–comfortable", "feel restless–feel calm", "artificial–natural", "feel coziness–not feel coziness", "bad odor–good odor". The bars represent the VAS scores in the Japanese cedar (black) and control conditions (white). Compared with the control condition, the score for "bad odor–good odor" under the Japanese cedar condition was higher ($P < 0.05$). *Statistical significance ($P < 0.05$). Data are shown as means ± SEM.

were defined by the average of the values obtained at 5-min intervals (Fig. 5). Compared with the control, the ratios of LF/HF tended to be lower at 10 min into the work period in the Japanese cedar condition. The values were 80.7 ± 7.8 (%) in the Japanese cedar condition and 120.1 ± 21.5 (%) in the control condition ($P < 0.1$). Slightly lower ratios of LF/HF were obtained at 15 min in the Japanese cedar condition ($P = 0.11$); 85.4 ± 10.5 (%) in the Japanese cedar condition and 138.3 ± 30.8 (%) in the control condition.

3.5. Salivary stress markers assay

Salivary stress markers were measured before and after each work period described in Fig. 2. Changes in salivary AA and CgA were observed. The salivary AAs were shown as the change from the pre-work measurement to the post-work measurement. The values of salivary AAs were −3.7 ± 6.3 (KIU/L) in the Japanese cedar condition and 13.8 ± 6.5 (KIU/L) in the control condition; this difference was tendency ($P < 0.1$) (Fig. 6a). In the Japanese cedar condition, the mean salivary CgAs were 1.0 ± 0.3 (ng/mL) at the pre-work measurement and 1.2 ± 0.2 (ng/mL) at the post-work measurement. In the control condition, the means in the post-work measurement were 0.9 ± 0.2 (ng/mL) and 1.7 ± 0.3 (ng/mL). This difference in the control condition was significant ($P < 0.05$) (Fig. 6b). No difference between the conditions was observed in the other stress markers, i.e., IgA and cortisol.

4. Discussion

In the present study, we found that VOCs in the Japanese cedar experimental room suppressed the activation of sympathetic nervous activity during and after arithmetic work. These findings indicate that VOCs emitted from Japanese cedar assist people in remaining physiologically relaxed under stressful conditions. Rooms with VOCs provided by wood are useful to maintain the balance of the autonomic nervous system. Therefore, such rooms have the potential to prevent mental health disturbances such as sleep disorders, restlessness, and depression.

The low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) components in ECG recordings are used to assess the degree of input to the heart from the autonomic nervous system: HF components and the LF/HF ratio reflect parasympathetic and sympathetic nervous activities, respectively. Salivary stress markers

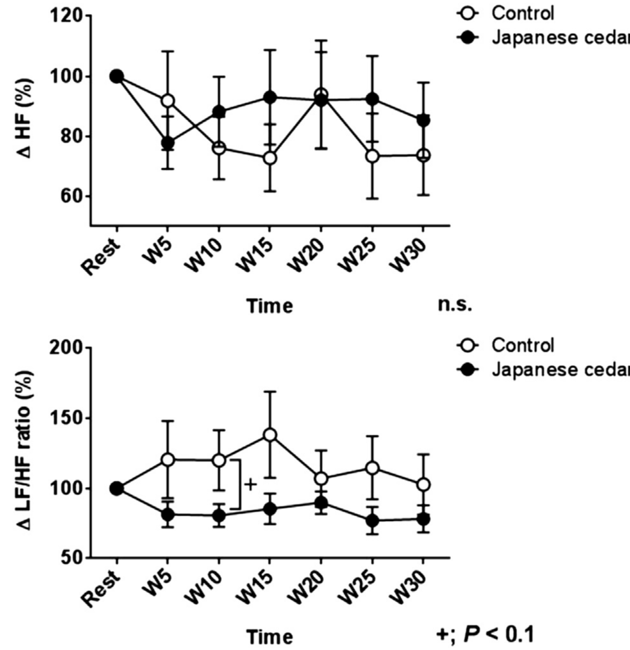

Fig. 5. HF components and LF/HF ratios during work and pre-work rest periods. Spectral analysis of two major components, the ratio of low frequency to high frequency (LF/HF, 0.04–0.15 Hz/0.15–0.4 Hz) and high-frequency (HF, 0.15–0.4 Hz) components are shown. W, 30-min work period divided into 5-min segments. Circles represent HF and LF/HF in the Japanese cedar (black) and control conditions (white). Compared with the control condition, the LF/HF in the Japanese cedar condition was significantly lower at 10 min into the work period ($P < 0.1$). + indicates tendency ($P < 0.1$). Data are shown as means ± SEM.

are useful for evaluating stress conditions by a noninvasive, convenient sampling method [14,15]. α-Amylase is an enzyme that is released from the salivary glands controlled by the sympathetic nervous system [16,17]. Chromogranin A is an acidic glucoprotein that is produced by submandibular glands and secreted into saliva [18]. And CgA is also released along with catecholamines from sympathetic nervous endings [19]. The release of salivary stress markers also depends on psychological stresses [20,21] and according previous reports, salivary AA and CgA have been used as stress markers related to the activation of the sympathetic nervous system under stressful conditions. In this study, the increases in LF/HF ratio (Fig. 5) and salivary AA and CgA (Fig. 6a and b) during and after arithmetic work were observed only in the control experimental room. We suggested that VOCs in the Japanese cedar experimental room suppressed activation of sympathetic nervous activity, as with previous studies [6–8]. Cardiac function is controlled by the autonomic nervous system and decrease in heart rate variability (HRV) and increase in heart rate are caused by autonomic dysfunction. The previous report has indicated that exposure to VOCs leads to decreases in HRV indices [22]. According to our results, there were suggested that the inhalation of VOCs emitted from Japanese cedar has the efficacy against the autonomic nervous system function. In future studies, we should discuss that the relationship between the compounds and concentration of VOCs and the influences of HRV indices.

Work performance was determined by the total number of correct calculations. No difference in performance was observed between the Japanese cedar condition and the control condition (Fig. 3). The U–K test is widely used as a simple workload test that causes mental fatigue in participants. Our questionnaire indicated that the participants did not feel heavy fatigue in either room after the experimental period (data not shown). This suggested that the

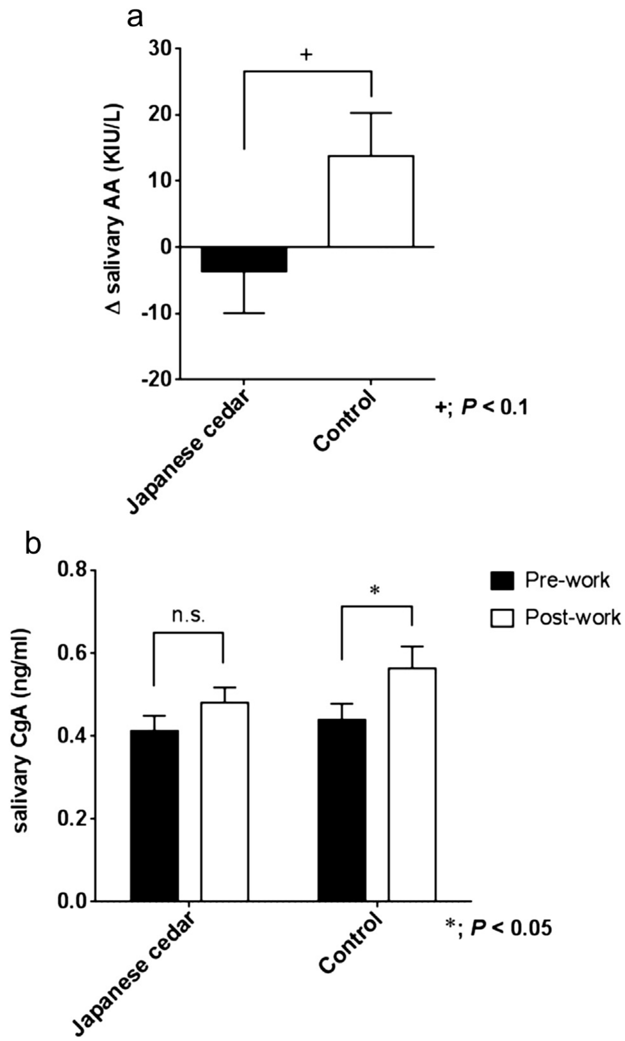

Fig. 6. a Variation of salivary α-amylase at pre- and post-work measurements. The bars represent the amounts of change in salivary α-amylase levels in the Japanese cedar (black) and control conditions (white). Compared with the control condition, the change in salivary α-amylase under the Japanese cedar condition was lower ($P < 0.1$) with tendency + indicates tendency ($P < 0.1$). Data are shown as means ± SEM. Fig. 6-b Variation in salivary chromogranin A at pre- and post-work measurements. Variations in salivary chromogranin A are shown. The bars represent the salivary chromogranin A levels in the Japanese cedar (black) and control conditions (white). Compared with the pre-work measurement under the control condition, the salivary chromogranin A at the post-work measurement was higher ($P < 0.05$). The change in salivary chromogranin A under the Japanese cedar condition between pre- and post-work measurements was not significant. * Statistical significance ($P < 0.05$). Data are shown as means ± SEM.

U–K test was applied without mental fatigue in our experiment. Therefore, task performance did not differ between the rooms.

The subjective effects of volatiles emitted from Japanese cedar differed significantly in one questionnaire item (Fig. 4). Our results showed that volatiles emitted from Japanese cedar cause participants to feel that the odor is pleasant. It is difficult to evaluate the relationships between subjective assessment and physiological responses and task performance. However, previous reports have indicated that the subjective effects of odorants influence task performance [9,23]. Additional experiments with different concentrations of volatiles are needed to clarify the subjective effects of odorants.

We identified and found that the volatiles emitted from Japanese cedar were δ-cadinene, α-muulorene, α-cubebene, β-cubebene and other sesquiterpenes. δ-Cadinene was the highest (Table 1). Our findings suggested the volatiles emitted from Japanese cedar and inhaled during the experiment suppressed sympathetic nervous activity and therefore prevented the increase in salivary AA and CgA (Fig. 6a and b) found in the control condition. The contribution of each volatile to this activity, the effect of their combination, and the optimal concentrations could not be discerned from our study design. Further experiments using each volatile are needed to clarify their modes of action. The emission of volatiles depending on the humidity and temperature in the environment are well known. We also should discuss the changes of psychophysiological responses by the long-term use of interior wall.

Wood has been used since ancient times as a building material around the world. One of the characteristics of wood is hygroscopic conditioning, by which the moisture content in wood changes depending on the change in room temperature and relative humidity in the environment [24]. Therefore, the present and previous results together suggest that an interior containing wood can help people maintain an optimum living environment.

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