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A pilot study on physiological relaxation and enhanced work performance by volatile organic compounds emitted from Kagawa hinoki (*Chamaecyparis obtusa*) interior walls

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Abstract

Since ancient times, Japanese cypress timber (*Chamaecyparis obtusa*; 'hinoki' in Japanese) has been highly valued in Japan. Because of its beautiful color and fragrance, this timber has been widely used as houses and furniture materials. Scientific evidence on the effect of the usage of hinoki's timber especially from Kagawa's area as a building material toward human psychophysiological response has yet to be reported. The current study examined participants' psychophysiological responses to volatile organic compounds (VOCs) generated by hinoki interior walls growing in Kagawa Prefecture, Japan. This timber was reported to have a relaxing effect toward the participants, and this study discovered that inhaling air containing VOCs emitted by Kagawa hinoki interior walls increased parasympathetic nervous activity during resting periods between arithmetic tasks. When compared to a control condition, the participants' performance on a calculation test improved dramatically in the hinoki condition. These findings suggest that VOCs released by Kagawa hinoki walls influence the autonomic nervous system, emotions, and work performance. This study not only proved Kagawa hinoki fragrance's potential to boost human working speed, but it also newly confirmed the fragrance's ability to increase parasympathetic nervous system activity while at rest. This study also discovered that the enhanced working speed is caused by a part of the neurological underpinning for heightened arousal in the brain. As a result, this study concluded that the aroma of cypress can have physiological effects that change over time, hence optimizing individuals' behavior at work and at rest.

Keywords Kagawa hinoki, VOCs, CG-MS, ECG, EEG, Relaxation, Work performance

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Introduction

Recent technological advances in the uses of timber have significantly increased the popularity of wooden buildings, interior walls comprised of or containing timber [1], landscaping with timber [2], structural engineering using timber [3], and timber furniture design [4]. The use of timber products is encouraged due to their dual capabilities: not only do they continue to store the carbon dioxide that trees have absorbed and sequestered from the atmosphere during their growth process; they also have the ability to absorb or release moisture from the air [5]. As trees grow, they release secondary metabolites of

terpenoids (primarily monoterpenes and sesquiterpenes) with high volatility and aromatically. This characteristic improves the quality of indoor air and promotes emotional stability in humans, thereby contributing to stress alleviation [6, 7].

Among the many species of trees used for their timber, Japanese cypress (*Chamaecyparis obtusa*; 'hinoki' in Japanese) is a typical coniferous tree species that is widely grown in Japan. Two *Chamaecyparis* species, i.e., *C. obtusa* and *C. pisifera*, are extensively cultivated in Japan as valuable timber resources [8]. These species exhibit high-quality characteristics and together account for ~14% of the total timber production volume in Japan [9]. The qualities of hinoki are known to be influenced by climatic and soil conditions, and the hinoki grown in Japan's Kagawa Prefecture, where the precipitation is relatively low, possesses a unique and pleasant strong fragrance.

Hinoki and its volatile organic compounds (VOCs) have been reported to exhibit significant effects, such as enhancing markers of immune system function in humans [10] and promoting physiological relaxation [11]. Hinoki produces and emits a diverse array of VOCs, primarily monoterpenes and sesquiterpenes, through precise and complex biosynthetic processes involving the hinoki's biological metabolism [12, 13]. The emissions of VOCs from hinoki are higher compared to other species such as Japanese cedars, larch, and meranti, and the composition of hinoki VOCs differ from those emitted by other tree species [13]. Hinoki is known to emit higher levels of the VOC α -pinene in particular [14, 15], and an anti-anxiety activity of α -pinene has been described [16]. In addition, hinoki essential oil has been used as an aroma material.

Matsubara et al. reported that VOCs in an experimental room lined with Japanese cedar (*Cryptomeria japonica*) suppressed the activation of participants' sympathetic nervous activity during and after arithmetic work, and they proposed that VOCs emitted by Japanese cedar help people remain physiologically relaxed under stressful environmental conditions [6]. However, their results suggested that the VOCs from Japanese cedar had no effect on the number of correct calculations or working speed on arithmetic tasks [6]. Regarding the physiological effects of VOCs on humans, exposure to essential oil made from hinoki cypress leaves induced physiological relaxation and resulted in a significant reduction of the oxygen-hemoglobin (oxy-Hb) concentration in the right prefrontal cortex and increased parasympathetic nervous activity [17, 18].

However, the physiological and neuroscientific neural bases of the effects of Japanese cypress (*Chamaecyparis obtusa*) fragrance have not been established. The effects

of hinoki odor on the physiological and brain mechanisms of both physiological relaxation and increased working speed should be further examined. Watanabe et al. used the content ratio of the power for the α -wave bands and the β -wave bands to compare the working speed in the number of correct answers in handedness during the Uchida-Kraepelin (UK) test, which is a challenging examination [19]. They observed a correlation between the α/β wave and the total score on the UK test. In addition, features of electroencephalography (EEG) that correlated with working speed were described by Fernandez Rojas et al. [20]: the value engagement index (EI), the task load index, and the θ/β ratio. For instance, the value obtained by $\beta/(\alpha + \theta)$ is known as the engagement index, which has been used to research mental effort, mental attention investment, alertness, and task engagement [21–23]. The θ/α ratio, also known as the task load index (TLI), is another measure used to investigate workload evaluation. This statistic is based on the idea that when mental strain increases, the α power in the parietal lobe declines and the θ power in the frontal lobes increases [23–25].

Thermal desorption unit/gas chromatography–mass spectrometry (TDU/GC–MS) has been used in studies investigating the psychophysiological effects of VOCs derived from various timber species. The authors of this study group are also conducting research on VOCs from timber [6, 26, 27]. In the present study, the chemical quantification of the concentration of VOCs emitted from hinoki timber was conducted to gain a better understanding of the characteristics and utility of hinoki as an interior material. The underlying mechanisms, from a psychological perspective, that contribute to hinoki VOCs' health effects on humans has been investigated. Participants completed the UK test, and simultaneously, EEG and electrocardiography (ECG) recordings were measured to investigate differences in the participants' workload performance based on their physiological activities, between a room constructed with hinoki cypress material and a control room with cloth walls without hinoki cypress material. Results on this study clarified the psychophysiological effects on the human body attributed to VOCs emitted from hinoki timber. This study is the first report to examine the effects of a room made with hinoki timber on the performance of the UK test, including its effects on the autonomic nervous system and emotions.

Methods

VOC analysis

The interior-wall timber used in this study was from hinoki cultivated and harvested by Ouken Co. in (Takamatsu, Kagawa, Japan). The negative control interior

walls were created by Ouken Co. as a hinoki timber like photoprinted cloth material adhering to concrete panels. The VOCs were collected using two mini-pump (flow rate: 0.15 L/min, volume: 3.0 L, duration: 20 min, Shibata, Saitama, Japan) and the sorbent tubes with VOC traps of Tenax® TA glass TD tubes, fritted, outer diameter 6 mm × inner diameter 4 mm × length 60 mm, pre-conditioned (Gerstel, Mülheim an der Ruhr, Germany). The tubes were conditioned using the TC-2 Tube Conditioner (Gerstel) by the manner of the manufacturer's recommendations.

The internal standard (IS) benzaldehyde and tridecane were purchased from Sigma Aldrich Japan, Tokyo. To establish a calibration curve for the quantification of multiple sesquiterpenes, β -caryophyllene (Sigma Aldrich) was utilized, and the obtained results are expressed as β -caryophyllene equivalents. All other solvents were analytical grade and used without any further purification.

Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis was performed by an Agilent 7890 A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a quadrupole mass spectrometer, an Agilent 5975 C mass selective detector (Agilent Technologies), with a 30 m × 0.25 mm DB-5 ms capillary column (0.25 μ m film thickness, Agilent Technologies). The GC–MS system was equipped with additional instruments, such as a thermal desorption unit (TDU) (Gerstel) and a cooled injection system (CIS) (Gerstel). The initial temperature for desorption was 40 °C; it was held for 0.1 min and then raised to 220 °C at 720 °C/min and held for 3 min. Desorbed compounds were then cryofocused on the CIS cooled at –100 °C and held for 0.1 min with liquid N₂. At the end of the desorption, the CIS temperature was raised to 220 °C at 12 °C/s and held for 10 min for the injection of the compounds into the column. The oven temperature program was as follows: 60 °C for 3 min followed by increases to 150 °C at 20 °C/min and 235 °C at 5 °C/min, and the temperature was then held at this level for 0.5 min. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. The samples were injected in splitless injection mode.

The VOCs collected from the experimental room were tentatively identified by comparing the obtained GC–MS data with the NIST17 and W9N08 mass spectral database libraries and several earlier studies [13, 14, 28]. Since some of the VOCs were not commercially available, the all-VOCs concentrations in the air was calculated as β -caryophyllene equivalents to determine the total VOC contents (μ g/m³). The calibration curve was established using benzaldehyde as the internal standard, over the range of 1–100 μ g/mL for β -caryophyllene. For the quantification of VOCs during each experiment, 1

μ L of mixture of 100 μ L/L benzaldehyde and tridecane in acetone was added to the Tenax TA glass TD tubes. The VOC concentration of the compound was calculated from the ratio of the peak area of benzaldehyde to the peak area of the detected compound.

Participants and experimental design

The experimental design of the study involving human subjects was approved by the local ethics committee, faculty of agriculture of Kyushu University, and the study was conducted in accord with the Declaration of Helsinki. This study was approved on the grounds that there is no risk to the participants on both plant experiments and human experiments. It should be noted that this study complies with the relevant institutional, national and international guidelines and legislation. All procedures were performed in accordance with the approved guidelines. Four healthy male and four female university students (age: 22.1 ± 2.1 years (mean ± standard deviation) participated. The purpose and schedule of the experiments were explained, and written informed consent was obtained from all participants prior to the study's initiation. The consumption of alcohol or medications on the day before the experiment was prohibited, as was the consumption of caffeine on the day of the experiment.

Figure 1 shows the experimental design. Prior to conducting the visual event-related potential (ERP) experiment, two mini-pumps equipped with Tenax® TA glass TD tubes were installed in the experimental (i.e., hinoki) room to collect VOCs. For all experiments, to saturate a consistent level of VOCs in the experimental room, ventilation was initiated 15 min before the start of the experiment by activating the exhaust fan and opening the experimental room's door for ventilation. Subsequently, the experimental room was closed for 30 min with a handheld fan circulating the air after the exhaust fan was stopped.

The mini-pumps equipped with Tenax® TA glass TD tubes for the VOC collection were set to activate 10 min after a participant entered the experimental room and were retrieved after the completion of the experimental tasks. An ECG recording was done during the participant's arithmetic work and rest. Each participant performed the experiment twice: once in the absence (control room condition) and once in the presence (hinoki room condition) of VOCs emitted from the hinoki.

Arithmetic work

This study used the Uchida–Kraepelin (UK) test [29], which is a serial addition test that requires takers to perform calculations as quickly and as accurately as possible. Supplied with pre-printed paper containing 15 lines of

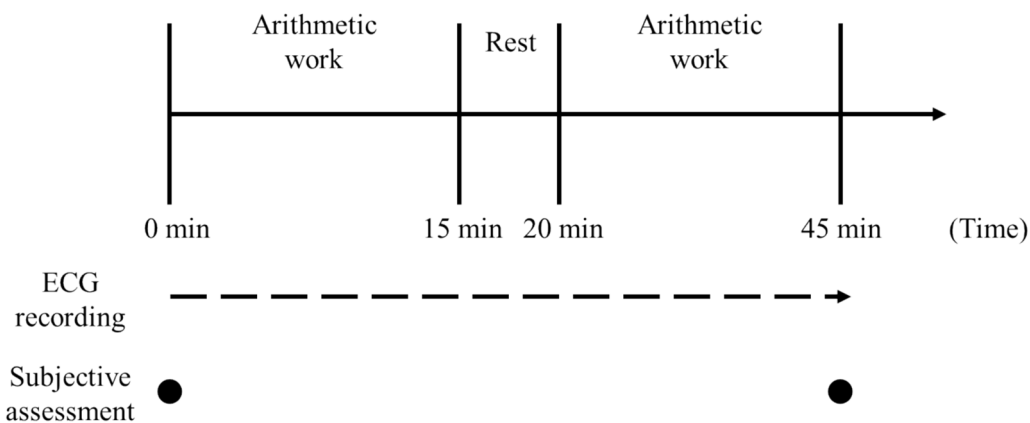


Fig. 1 Experimental protocol. Subjective evaluations of the fragrance were administered just before participants entered the experimental rooms and immediately after they left. The ECG recording was continuously measured throughout the participants' time in the experimental room

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 1 min. The test was performed for repeated cycles of 15 min of work and 5 min of rest.

Subjective evaluations of the fragrance of VOCs emitted by the hinoki walls

For the assessment of their overall subjective feelings associated with the fragrance, the participants were asked to fill out a questionnaire using a visual analog scale (VAS) and a semantic differential (SD) method [30]. The participants were given a VAS and instructed to identify the position of each word along a continuous line (0–100 mm) between the two endpoints. The participants were asked to make a mark on the line that fit their intuitive feeling in response to the room's fragrance. To investigate the distinct perceptions of the smell in the room, a questionnaire containing the following nine words was administered, each on a VAS of 0–100 mm: sweet, bitter, sober, sour, stink, perfumed, and fresh. The VAS scores were measured using a ruler. On the other hands, SD methods were based on earlier investigations [31–33], we used the following 15 pairs of adjectives in this study: like/dislike, clear/unclear, light/heavy, relaxed/not relaxed, natural/artificial, comfortable/uncomfortable, calm/tense, light/strong, old/modern, elegant/vulgar, pleasant/unpleasant, refreshed/unrefreshed, transparent/murky, urban/rural, and fresh/not fresh.

To assess the participants' mood states, the short form of the Profile of Mood States 2 (POMS-SF) was utilized [34]. The participants were asked to check a five-point scale ranging from 'not at all' to 'extremely' for each of the 30 questions on the POMS-SF before leaving either room. The POMS-SF questions are assigned to

six different subscales of mood: tension–anxiety (T–A), anger–hostility (A–H), vigor (V), fatigue (F), depression (D), and confusion (C). We calculated T-scores according to the POMS manual, and mood states were assessed quantitatively [34]. For the identification of each participant's preferences, each was asked to disclose their preferred room and to state the reasons why they preferred the room after they had finished their stays in both the hinoki and control rooms.

ECG recordings and analysis

ECG signals were recorded simultaneously using a Poly-mate V AP5148 bio-signal recording device (Miyuki Giken, Tokyo) at a sampling rate of 1000 Hz with the use of three active electrodes. The noise in raw ECG data due to muscle tone, blinking, and other small movements, were removed by the display software, AP Viewer. One male participant's data were excluded, because the correct ECG signal was not measured due to equipment trouble. After the detection of R-peaks, the R–R intervals (RRI) by subtracting the peak time from the previous timing were measured to form the waveform that shows the temporal alternation of the peak timings. In the analysis, data with a window size of 60 s and a step size of 1 s were used to obtain the RRI spectrogram. Using the code demodulation method, the power values of the low-frequency (LF) (0.04–0.15 Hz) and high-frequency (HF) (0.15–0.4 Hz) amplitudes were calculated.

EEG recordings and analysis

The data were collected on different days between testing in the control room and the room with hinoki cypress timber materials. Two participant's data were excluded, because the correct EEG signals were not measured due to equipment trouble. The trial in the control room and

the trial in the hinoki room were held on different days, and to avoid the ordering effect, a counterbalance was applied. For the measurement of the participants' brain activity, the EEG during the UK test was recorded. The EEG electrodes were placed on the participant's frontal midline (Fz) and occipital midline (Oz) with two references: the linked-ear lobe (LR) and the nasion. The frontal Fz electrode was placed to record Fm θ . The background EEG activity was recorded at the Oz electrode to distinguish between Fm θ and occipital θ caused by drowsiness. Each participant was asked to take the UK test in two sessions, with a rest period between sessions. The test was conducted for a 15-min period in each session, and the rest period was 5 min.

Custom MATLAB scripts were developed for the data analyses. The raw data during the experiment were then filtered with the use of a notch filter and a bandpass filter with a [0.05–30] Hz cutoff frequency. The signal with an amplitude > 80 μ V were defined as movement artifacts. Therefore, they were removed, and interpolation was applied. MATLAB periodogram function and band power function was used to calculate the power spectral density on each band. The content ratio of each band was then calculated, as shown in Eqs. 1–3 [19], where S_n is the power spectral density of the signal at frequency n , and C is the content ratio. α band is calculated in frequency range between 8 to 13 Hz, θ band is calculated in the frequency range 8–4 Hz, β band is calculated in the frequency range 13 to 30 Hz. The engagement index ($\beta/(\alpha + \theta)$) represents the ratio of β band toward other slow wave ($\alpha + \theta$), which correlation with the ability of participant on keep engaging on the task was then calculated based on Eq. 4 [20]. We also calculated the θ/β ratio which mentioned to be used as the monitor of sleepiness in Eq. 5 [20]. Those equation could reduce the effect of subjective variations, because the calculation is based on the relative ratio instead absolute value. Finally, Pearson's correlation between the index and the total score on the UK test was calculated with Eq. 6, where r is the correlation coefficient and x_i is the value of the UK total score workload, while $\bar{x}$ is the mean value. In the same fashion, y_i represents the investigated value of EEG features in a sample, and $\bar{y}$ is the mean value of the EEG features:

$$C_\alpha = \frac{\sum_{n=8}^{13} S_n}{\sum_{n=1}^4 S_n + \sum_{n=4}^8 S_n + \sum_{n=8}^{13} S_n + \sum_{n=13}^{30} S_n} \times 100[\%] \quad (1)$$

$$C_\theta = \frac{\sum_{n=4}^8 S_n}{\sum_{n=1}^4 S_n + \sum_{n=4}^8 S_n + \sum_{n=8}^{13} S_n + \sum_{n=13}^{30} S_n} \times 100[\%] \quad (2)$$

$$C_\beta = \frac{\sum_{n=13}^{30} S_n}{\sum_{n=1}^4 S_n + \sum_{n=4}^8 S_n + \sum_{n=8}^{13} S_n + \sum_{n=13}^{30} S_n} \times 100[\%] \quad (3)$$

$$\text{Engagement index} = \frac{C_\beta}{(C_\alpha + C_\theta)} \quad (4)$$

$$\frac{C_\theta}{C_\beta} = \frac{\theta \text{ power component}}{\beta \text{ power component}} \quad (5)$$

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \quad (6)$$

Statistical analyses

All results are expressed as the mean $\pm$ SEM (standard error of the mean). To compare differences in subjective assessments between the conditions, a paired t test or Wilcoxon's signed-rank test was conducted. We performed a two-way analysis of variance (ANOVA) to examine the HF and LF/HF ratio data. ANOVA followed by Bonferroni post hoc tests was conducted using all the subjects' data. Statistical significance was recognized when the p value was < 0.05, and a tendency was defined as a p value < 0.1). All statistical analyses were performed using R (ver. 4.3.1) and JASP ver. 0.17.2.1.

Results

Identification of the VOCs emitted from hinoki

The GC chromatogram of VOCs emitted in a room with hinoki interior walls in the absence of participants is provided in Fig. 2A, B. The peaks shown in the figure are derived mainly from natural terpenes emitted by the hinoki interior walls. Based on the results of the GC–MS analysis, the Japanese cypress (hinoki) walls contained 21 major VOCs, which were classified into four distinct classes (olefine monoterpenes, monoterpene alcohols, olefine sesquiterpenes, and sesquiterpene alcohols), each containing a variety of compounds, including major constituents, such as α -pinene, δ -cadinene, α -muurolene, γ -muurolene, β -elemene, γ -cadinene, and others, as listed in Table 1. As a result, the total concentration of VOCs in the uninhabited hinoki room was significantly higher than in the room with simulated cypress cloth wallpaper. Regarding the small number and amount of VOCs (such as α -pinene, β -myrcene, d-limonene, etc.) and 2-ethyl-1-hexanol detected in the control room, we speculated that they originated from the framework securing the interior materials or from plasticizers. None of the VOCs detected in this study have been labeled as harmful VOCs by Japan's Ministry of Health, Labor, and Welfare (MHLW) in either the hinoki room or control room.

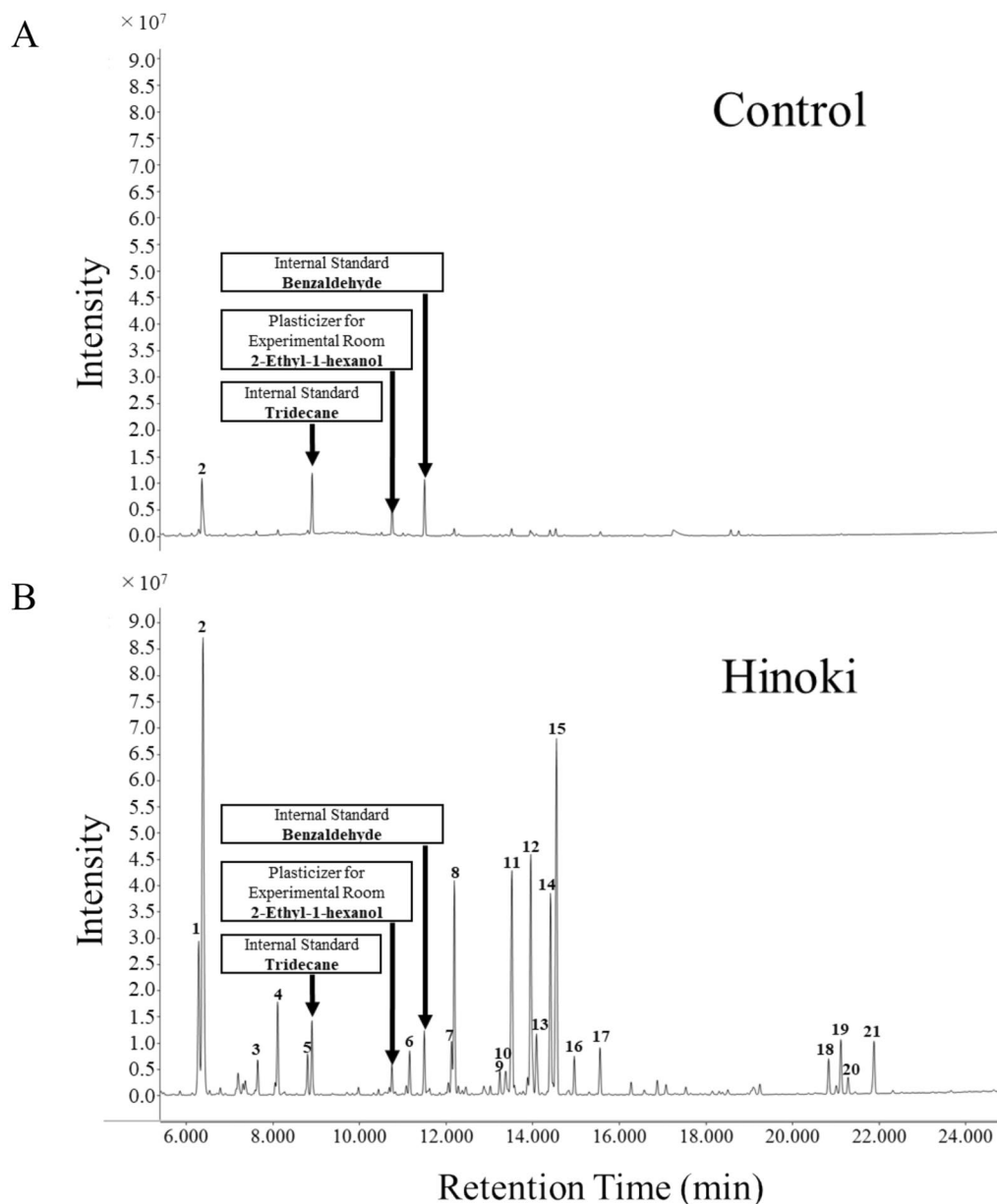


Fig. 2 GC spectrum of VOCs collected from the experimental rooms using a Tenax TA tube. Upper: **A** cloth interior walls. **B** Hinoki interior walls

Arithmetic (Uchida–Kraepelin) performance

The participants performed the UK test [29] during the work period and remained quiet during the rest period in the experimental room shown in Fig. 3A, B. The experimental room in our university (Inside dimensions: width 1700 mm $\times$ depth 1260 mm $\times$ height 1960 mm) was used. This test is a serial addition test that requires takers to perform calculations as quickly and as accurately as possible. The test was performed for repeated cycles of 15 min of work period and 5 min of rest period. The participants' performance on the UK

test was evaluated by the total number of correct calculations made, which was calculated as the sum of correct answers during the 15-min work period. Specifically, the total number of correct answers per minute for each participant was counted, and the average over the 15-min period was calculated to determine the total number of correct answers. The number of correct calculations were significantly higher under the hinoki condition than the control conditions ($t(7) = 6.470$, $p < 0.001$) (Fig. 4).

Table 1 Volatile organic compounds (VOCs) and their concentrations identified in this study

No	RT	Tentatively identified compound	Cloth interior walls, mean $\mu\text{g}/\text{m}^3$	Hinoki interior walls, mean $\mu\text{g}/\text{m}^3$
1	6.28	Unidentified	–	59.48 ± 5.16
2	6.38	α -Pinene	23.74 ± 5.29	233.90 ± 16.11
3	7.64	β -Myrcene	2.92 ± 0.41	11.04 ± 0.60
4	8.10	<i>d</i> -Limonene	3.45 ± 0.17	31.97 ± 1.39
5	8.79	Cymene	3.30 ± 0.09	14.60 ± 0.73
6	11.16	Copaene	2.38 ± 0.19	14.40 ± 0.77
7	12.12	Bornyl acetate	2.70 ± 0.05	19.18 ± 0.79
8	12.18	β -Elemene	4.69 ± 0.24	71.47 ± 1.28
9	13.24	<i>cis</i> -Verbenol	2.44 ± 0.12	8.22 ± 0.30
10	13.37	β -Gurjunene	2.76 ± 0.03	9.13 ± 0.09
11	13.52	γ -Murolene	4.62 ± 0.28	84.33 ± 1.61
12	13.95	α -Murolene	3.93 ± 0.18	96.82 ± 2.12
13	14.09	β -Selinene	2.81 ± 0.13	23.10 ± 0.10
14	14.41	(<i>R</i>)- γ -Cadinene	4.16 ± 0.15	73.32 ± 5.25
15	14.55	δ -Cadinene	4.66 ± 0.21	143.41 ± 0.78
16	14.96	α -Cadinene	2.31 ± 0.07	14.36 ± 0.22
17	15.55	(1 <i>S</i>)- <i>cis</i> -Calamenene	3.57 ± 0.36	18.52 ± 0.20
18	20.83	τ -Cadinol	2.09 ± 0.02	14.91 ± 0.05
19	21.11	τ -Murolol	2.39 ± 0.05	22.20 ± 0.41
20	21.29	(+)-Cedrol	2.16 ± 0.02	1.94 ± 0.01
21	21.88	α -Cadinol	2.16 ± 0.07	23.88 ± 0.63

RT: retention time

Figure 5 depicts each participant's time-series change of correct calculations, while they were solving the UK test's arithmetic problem. S in the label is stand for Subject. The number of additions per minute in the second session (red line) is higher than first session (blue line), this is attributed to the initial effects such as pressure and how to solve the UK test during the first session [35]. The answer for the most participant under the hinoki condition was higher than that for control except for one of the eight participants (S8).

Analysis of subjective evaluations of the fragrance of VOCs emitted from hinoki by questionnaire

For the assessment of their overall subjective feelings associated with the fragrance, the participants were asked to fill out a questionnaire using VAS score and SD methods [30]. In addition, to assess the participants' mood, the short form of the Profile of Mood State 2(POMS-FS) was used [34]. The participants' VAS scores were different between the hinoki and control conditions: the rating 'perfumed' was significantly increased in the hinoki room compared to the control room ($t(7) = -3.410$, $p = 0.011$). The SD scores showed significant differences between the hinoki and control conditions, as the following ratings were significantly higher in the hinoki condition compared to the control condition: 'like' ($t(7) = -3.415$, $p = 0.011$), 'clear' ($t(7) = -4.708$, $p = 0.002$), 'relaxed' ($t(7) = -3.549$, $p = 0.009$), 'comfortable' ($t(7) = -4.781$, $p = 0.002$), 'strong' ($t(7) = 4.460$,

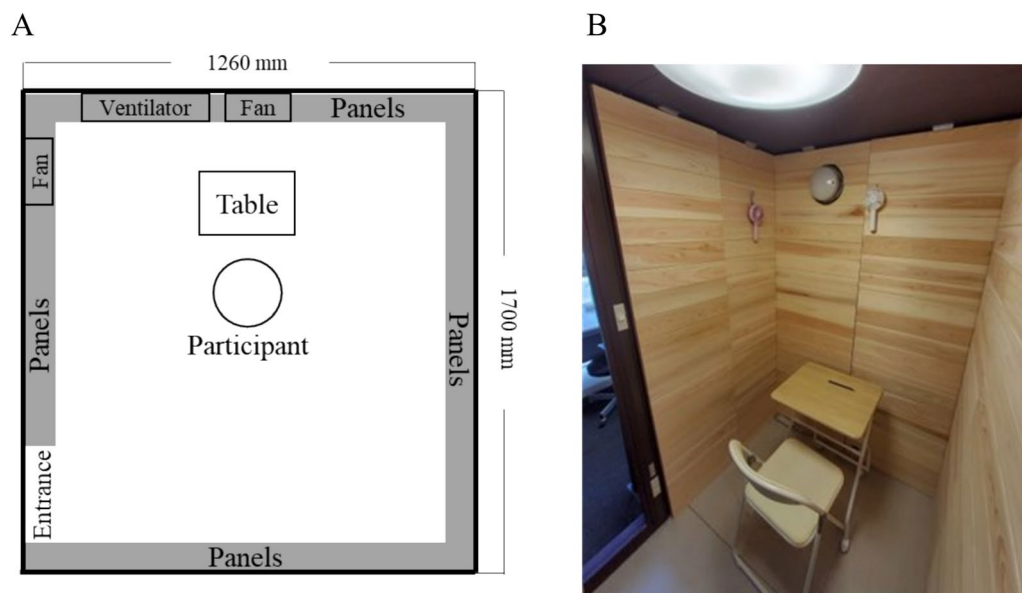


Fig. 3 **A** Schematic diagram of the experimental room. **B** Actual experimental room scene. The participants entered the room, sat facing the desk, performed arithmetic work during the work period, and then remained in their seats during the rest period. In the hinoki condition, hinoki wood panels were installed on the walls of the experimental room

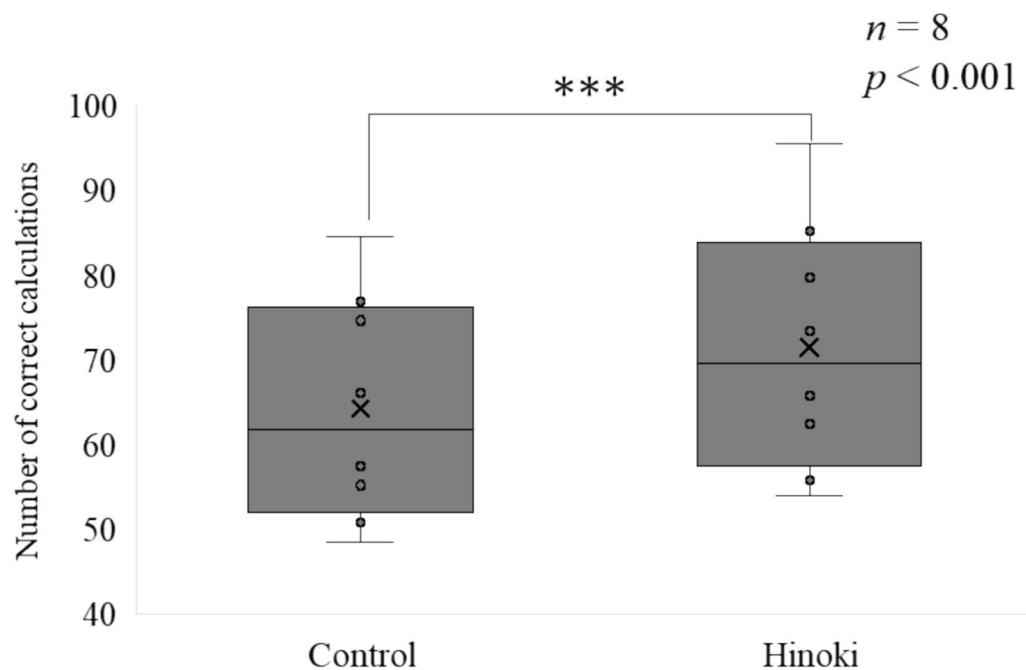


Fig. 4 Total number of correct calculations in arithmetic work (UK test). The bars represent the number of calculations in the control condition and hinoki condition. The number of correct calculations in the hinoki condition was significantly higher than in the control condition. The box represents the middle 50% of the data (from the first quartile to the third quartile). The upper part of the box indicates the third quartile (the top 25% of the data), while the lower part indicates the first quartile (the bottom 25% of the data). The central line within the box represents the median. The whiskers show the range of the data. The “x” inside the box represents the mean value. Statistical significance ** $p < 0.01$, * $p < 0.05$

$p = 0.002$), ‘pleasant’ ($t(7) = -5.291$, $p = 0.001$), and ‘transparent’ ($t(7) = -3.968$, $p = 0.005$). The POMS2 scores were significantly different between the hinoki and control conditions: the ‘fatigue’ scores were significantly decreased in the hinoki condition compared to the control condition ($t(7) = 2.997$, $p = 0.024$).

Analysis of the participant’s cardiac autonomic nervous activity during the arithmetic work

ECG recordings was performed before and after each work period and is shown in Fig. 1. To analyze the participants’ autonomic nervous activity by determining the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) components. After the detection of R-peaks, the R–R intervals (RRI) are calculated by subtracting the peak time from the previous timing to form the waveform that shows the temporal alternation of the peak timings. In the analysis, a window size of 60 s has been used and a step size of 1 s to obtain the RRI spectrogram. The values of the HF components and the LF/HF ratio were obtained as the average of the values recorded at 5 min intervals. One male participant’s data were excluded, because the correct ECG signal was not measured due to equipment trouble. In general, LF/HF is known to reflect sympathetic nervous system activity, while HF reflects parasympathetic nervous system

activity [36]. Figure 6A shows that there was no statistically significant difference in LH/HF values between the control condition and the hinoki condition. In contrast, Fig. 6B depicts that the resting HF value was significantly higher in the hinoki condition compared to the control condition ($p < 0.001$, $n = 7$).

Analysis of the participants’ brain activity during the arithmetic work

EEG showing results of brain activity analysis are summarized in Fig. 7A. First, as shown in Fig. 7A, left, the results of our time–frequency analysis of the grand-averaged EEG power spectral density during series of UK-rest-UK revealed the different activities between resting and doing the tasks. α -Wave and θ -wave showed higher power during the rest period, whereas during the task session on the UK test, the β -wave showed higher power compared to that during the rest period.

Second, as shown in Fig. 7B, upper right, during the first session of the UK test, the majority of participants did not show any statistically significant differences between the total UK test score and the engagement index ($\beta/(\alpha + \theta)$), suggesting mental effort in both the control and hinoki conditions. On the other hand, statistically significant trend in the engagement index (mental effort, mental attention investment, alertness) began to

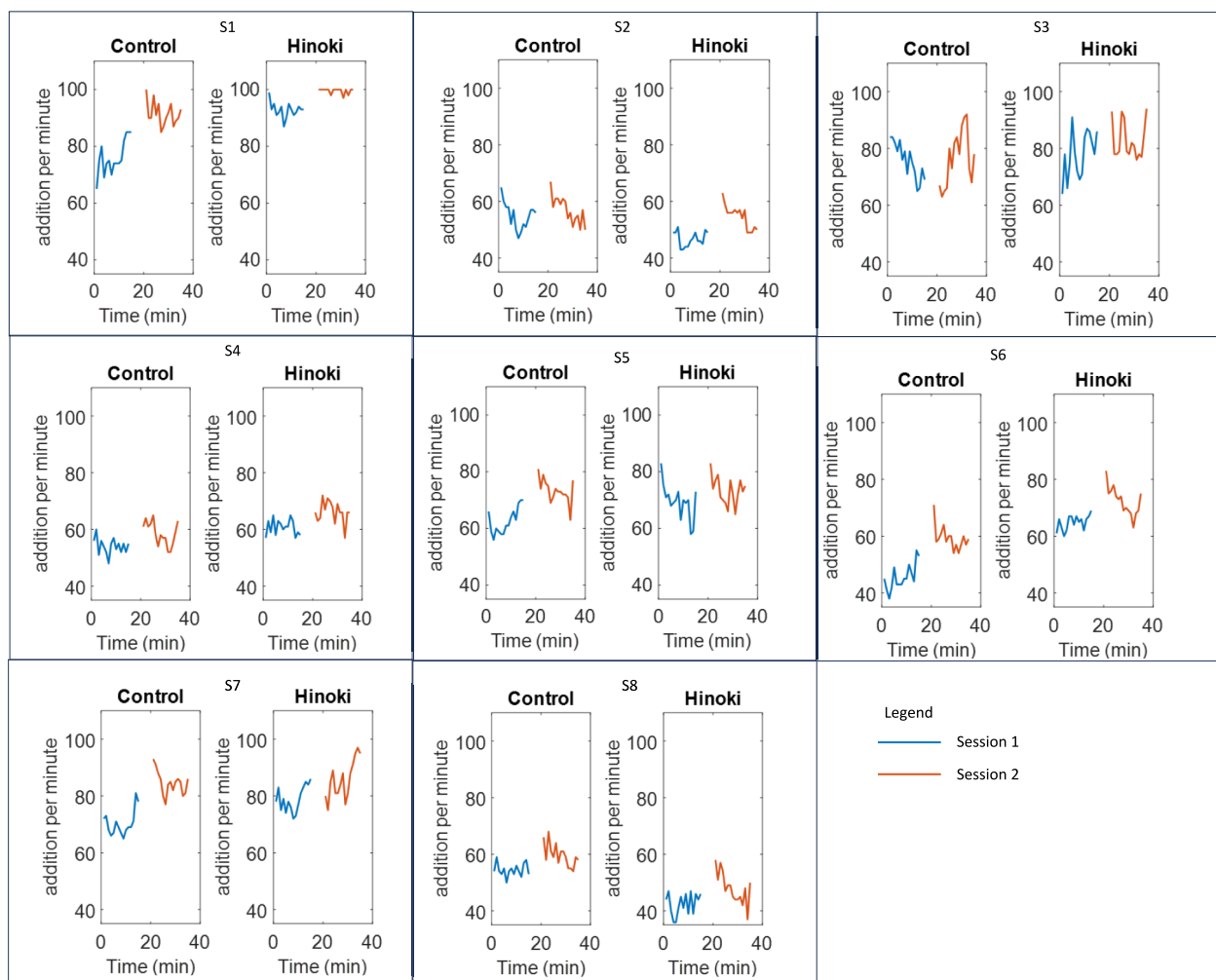


Fig. 5 Time-series changes in the number of correct answers in each 1 min in the UK task for the eight participants under the hinoki and control condition. The blue line indicates the total answers of the first half of the UK task; the red line indicates that of the second half. Six of the eight subjects obtained higher correct answers under the hinoki condition

be observed in session 2. The engagement index in session 2 under the hinoki condition, showed a slight tendency to have a positive correlation to the total UK test score. In contrast, in the control condition, the majority of participants in session 2 showed no correlation or a slightly negative correlation between the engagement index and the total UK test score. Only one participant in 6 participants showed a slight positive correlation toward the engagement index.

Third, as shown in Fig. 7C, lower right, the correlation of the UK test's total answer for additions per minute and the ratio of θ and β components in EEG showed negative correlation in session 1 in the control condition but no clear tendency of the θ/β ratio in the hinoki condition. However, in session 2, the θ/β ratio in the control condition started to have no correlation, whereas in the hinoki condition, there was negative

correlation between the θ/β ratio and the UK total score.

Finally, as shown in Fig. 7D, the θ/β ratio at the occipital region (Oz) in session 1 in the hinoki condition was significantly decreased compared to that in the control condition (Wilcoxon signed-rank test, $z=2.201$, $p=0.031$, effect size=1.00) (Fig. 7D, lower left). This decrease in the θ/β ratio suggested that hinoki's odor enhanced the participants' brain arousal levels in the first half of the sessions. Thus, part of the reason of the increased working speed can be affected by the arousal of brain activity in the hinoki condition.

Discussion

Kagawa hinoki timber emits predominantly α -pinene, δ -cadinene, α -muurolene, γ -muurolene, β -elemene, and other sesquiterpenes, with a notable abundance of

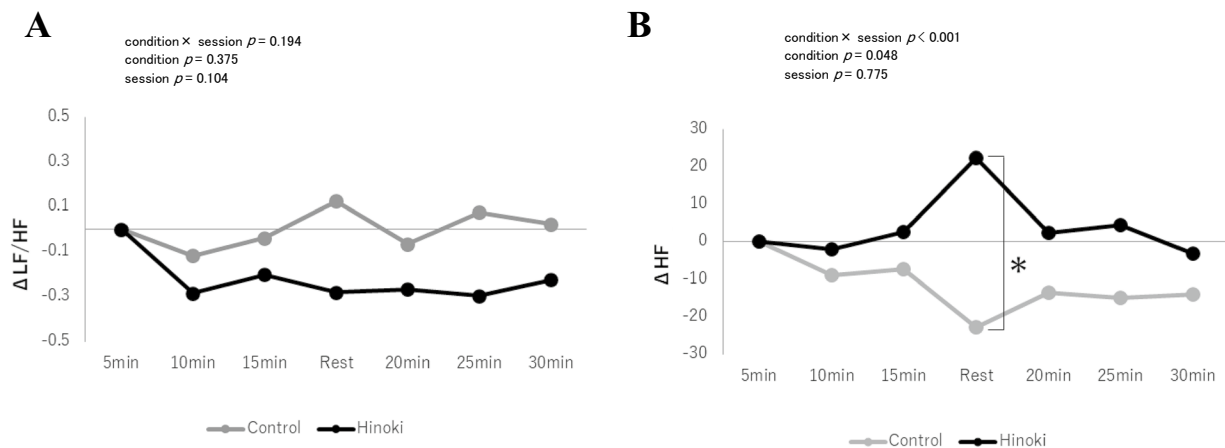


Fig. 6 **A** LF/HF during arithmetic work. **B** HF during arithmetic work. The lines represent the temporal changes in the change values calculated based on the data recorded in the first 5 min after the start of the task. The control condition represented by the gray line, and the hinoki condition is represented by the black line. In the HF, during the rest period, the hinoki condition showed a significant increase compared to the control condition

α -pinene and δ -cadinene release (Table 1). These VOCs can be regarded as a distinctive characteristic of ‘Kagawa hinoki’ compared to literature on other cypress tree species [13, 28, 37]. Moreover, it has been reported that variations in rainfall and moisture conditions influence the stomatal conductance of cypress, leading to fluctuations in the emission of VOCs [38]. Given that Kagawa Prefecture is one of the regions in Japan with the lowest annual precipitation [39], we consider that Kagawa hinoki grown in this area exhibit distinct characteristics and emit a more pronounced and unique fragrance compared to those from other regions.

This study present analyses revealed that VOCs in the hinoki experimental room increased the activation of parasympathetic nervous activity during the rest period between the arithmetic work periods. These findings indicate that VOCs emitted from Kagawa hinoki help promote physiological relaxation during rest under stressful conditions. The low- and high-frequency components in ECG recordings are used to assess the degree of input to the heart from the autonomic nervous system; the HF component reflects parasympathetic nervous activity, and the LF/HF ratio reflects sympathetic nervous activity [36]. In this study, no significant difference in the LF/HF ratio was observed between the hinoki condition and control condition during the arithmetic work and during the rest between arithmetic work (Fig. 6A). As in an earlier investigation, VOCs in the present hinoki condition suppressed the activation of sympathetic nervous activity [40]. Our present findings indicate that the inhalation of VOCs emitted by Kagawa hinoki has efficacy against the autonomic nervous system’s function.

Participants’ work performance was determined by the total number of correct calculations. Significant differences in the performance were observed between the hinoki and control conditions (Fig. 4). The UK test is used as a simple test of working speed that causes mental fatigue. However, it has not been clear whether or not the UK test per se provides psychological/mental stress to its taker [41]. According to the present participants’ subjective assessment of VOCs emitted by Kagawa hinoki, they felt that the hinoki odor is liked, clear, comfortable, strong, pleasant, clear, and relaxed. Other investigations have indicated that the subjective effects of odorants influence task performance [42, 43]. This study concludes that volatiles emitted by Kagawa hinoki can induce pleasant and relaxing emotions, and this effect may be particularly effective during rest breaks between arithmetic tasks.

The effect of the hinoki room on the participants’ working speed and its correlation with physiological activity has also been evaluated. The UK test has been used for inducing a mental workload, since the UK test was conducted in two sessions (Session 1, Session 2) with rest time between them. The percentage of component power on the EEG band was employed to reduce the confounding factors due to individual differences and complexity.

One of the focuses of this study was to clarify the brain activity in the frontal area, since this area correlates with decision-making, problem-solving, and attention [17, 44]. The results clarified that the engagement index has a positive correlation with the workload on the UK test’s performance in the hinoki room, whereas the θ/β ratio and α/β ratio showed a negative correlation, especially in session 2. This brain activity during experiment is

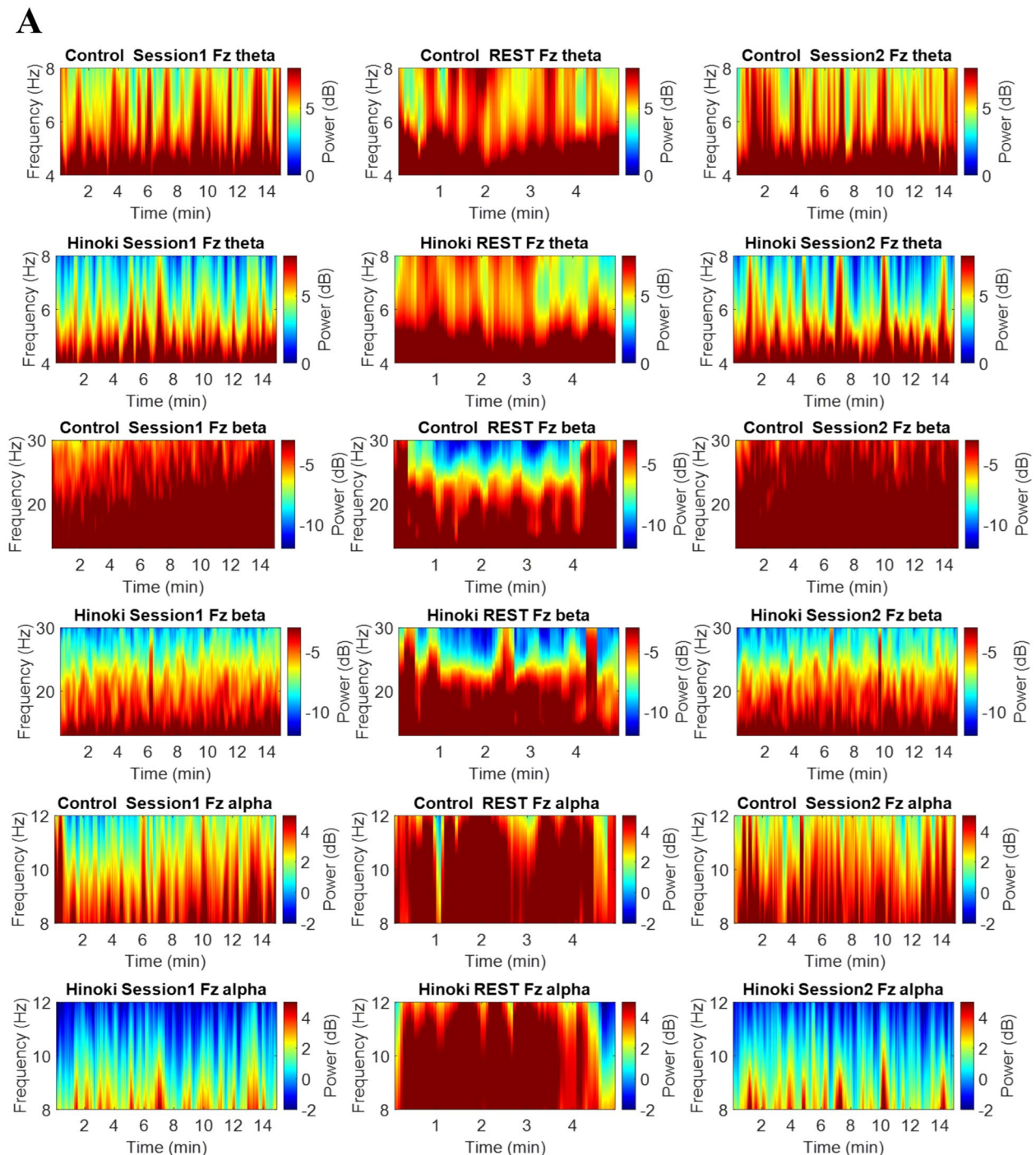
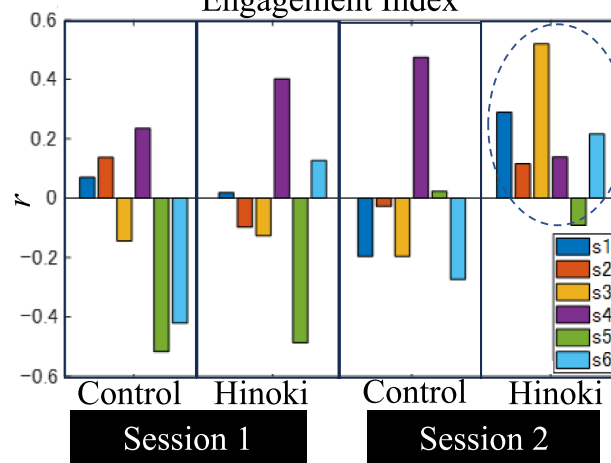


Fig. 7 **A** Grand average of EEG spectrogram of α band, β band and θ band during the experiment performance both in control and Hinoki's room. During the rest during the UK tests showed the higher power of α -wave and θ -wave, whereas during the task session on the UK test, the β -wave showed higher power compared to that during the rest period. **B** Engagement Index and the correlation with the number of successful UK responses. In Session 2 under the hinoki condition, positive correlations were found in 5 of 6 participants, while negative correlations were found in 5 of 6 participants. **C** θ/β and the correlation with the number of successful UK responses. Negative correlations between θ/β and the number of correct responses to UK were observed in 5 out of 6 participants in Session 2 under the hinoki condition (bottom right). **D** θ/β at occipital region (Oz) during each session under each Hinoki and control condition. Under the hinoki condition, θ/β was significantly reduced in all participants in session 2 from session 1, suggesting hinoki's fragrance can induce the increased arousal levels in the brain

B Correlation value (r) addition per minute and Engagement Index



C Correlation value (r) addition per minute and $\frac{\theta}{\beta}$

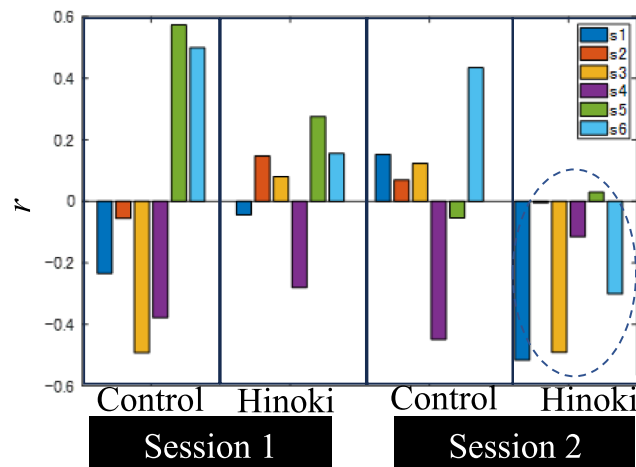


Fig. 7 continued

also emphasized by the results provided in spectrogram of EEG in α , β and θ , as shown in Fig. 7A. The β power increased as the θ power declined, indicating a high level of attentiveness among the participants. A decrease in β power and an increase in θ power signify a state of tiredness or low vigilance [18]. The α/β ratio shows a tendency to have a negative correlation with the total score on the UK test. Watanabe et al. reported that there is also a correlation between the α/β ratio and the total score on the UK test [19]. In the present study, the α/β wave showed a negative correlation with the UK test score; this could be due to the participants' high level of attentiveness to the task. At the same time, EI $\beta/(\alpha + \theta)$ has been used

to evaluate mental effort, mental attention investment, alertness, and task engagement [24, 25]. The present EI and θ/β ratio data suggest that our participants were more closely engaged in the task when they were in the hinoki room compared to when they were in the control room.

As mentioned above, the EI has been used as a parameter of increased mental strain, and it was reported to be associated with a decline in α power at the parietal/occipital area and an increase in θ power at the frontal midline area [8–10]. The data in session 2 revealed a negative correlation between the total score on the UK test and the engagement index in the hinoki room and a

tendency for a positive correlation in the control room. This result led to our speculation that the participants felt less stress during the test in the hinoki room. In addition, most participants showed increases in correct answer after the rest in hinoki room. This study suspects that the hinoki room could enhance the pause effect, resulting in an increased working speed [21, 22, 45].

Finally, the θ/β ratio, which is an EEG index indicating low arousal, was decreased in all of participants in the hinoki room during the first half of the task. In other words, the results indicated that arousal increased as the participants began to smell the scent of Kagawa hinoki. This effect on brain function may also be one of the factors contributing to the increase in work speed.

This study results are not only confirmed an improved working speed due to the effect of Kagawa hinoki fragrance; but also newly confirmed the Kagawa hinoki fragrance's ability to increase parasympathetic nervous system activity during a resting state. Our findings indicated that part of the neural basis for increased arousal in the brain is responsible for the increased working speed. The scent of Kagawa hinoki can have physiological effects that vary over time to optimize behavior during work and rest. The functionality of natural product scents should be clarified not only in terms of their relaxing effects, but also in terms of their arousing effects. Our findings demonstrate that psychophysiological effects of Kagawa hinoki fragrance can vary depending on the effects' interaction with an individual's behavior.

The main limitation of this study is the small sample size. In addition, the participants were limited to male and female university students. To apply and generalize the results to the general population, further research with a larger sample size, including participants ranging from children to the elderly, is necessary.

Abbreviations

VOCs	Volatile organic compounds
UK	Uchida–Kraepelin test
EEG	Electroencephalography
EI	Electroencephalography
TLI	Task Load Index
TDU/GC–MS	Thermal desorption unit/gas chromatography–mass spectrometry
ECG	Electrocardiography
IS	Internal standard
SS	Syringe spike
TDU	Thermal desorption unit
CIS	Cooled injection system
ERP	Event-related potential
SD	Semantic differential
VAS	Visual analog scale
POMS-SF	Profile of mood states
RRI	R–R interval
LF	Low frequency
HF	High frequency
SEM	Standard error of the mean
ANOVA	Analysis of variance
MHLW	Ministry of health, labor and welfare

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Author contributions

FK and KT conducted all of the psychophysiological experiments. KT performed all of the VOC analyses. FK analyzed the behavioral performance, psychological evaluation, and ECG data. FZ performed the analyses of the EEG time–frequency and EEG indices. TN supervised the EEG and analyzed EEG data and conducted the behavioral experiment and its analysis. AI and KF supervised the GC–MS analysis. RT, HY, and NY prepared the natural Kagawa hinoki wood for the room construction and built the experimental rooms. NF provided advice about the wood materials. FK, KT, TN, FZ and KF wrote the manuscript. KS managed this project. All authors have read and agreed to the submitted version of the manuscript.

Data availability

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

This research was supported by Ouken Co. Ltd. Ouken Co. Ltd is a home construction company. RT, HY, and NY are employees of Ouken Co. Ltd. The other authors declare that they have no known competing financial interests or personal relationships.

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