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Volatiles emitted from the leaves of *Laurus nobilis* L. improve vigilance performance in visual discrimination task

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ABSTRACT

The leaves of *Laurus noblis* L. (laurel) are mainly used as a spice in cooking, and the essential oil obtained by steam distillation of the leaves is used as an additive in foods, drugs and cosmetics. We investigated the effect of the volatiles emitted from the leaves of *L. noblis* at different doses (low-dose and high-dose groups) on vigilance performance in a visual discrimination task. By inhaling volatiles of the leaves of *L. noblis*, the decrease of the rate of true hits found in the control group was prevented in the low-dose group. The high-dose group showed higher scores than the low-dose group for subjective effects related to negative emotion. Meanwhile, both groups showed physiological effects suggesting stimulation of circulation. These findings suggest that the volatiles emitted from the leaves of *L. noblis* at low concentration could be utilized to maintain a high level of vigilance performance, such as the rate of true hits by improving physiological arousal without incurring the detrimental performance effects of negative emotion.

Laurus nobilis L., known simply as "laurel" is an ornamental tree indigenous to the Mediterranean region, and is cultivated as an evergreen tree or shrub in many temperate and warm parts of the world (17). The dried leaves of *L. noblis* are used as a spice or flavoring agent in the culinary and food industries. The essential oil prepared from the leaves has been reported to have antibacterial, antioxidant and anti-inflammatory activities (5, 24). The leaves of *L. noblis* have been also used to treat rheumatism, neuralgia and scabies in Japanese folk medicine (14, 21).

The pharmaceutical and therapeutic potentials of aromatic herbs have been widely discussed, especially for the treatment of mental health issues such as anxiety, depression and sleep disorders (9). Recently, their application to working conditions has been discussed and investigated in relation to performance in a visual discrimination task (13, 27, 30). Vigilance tasks require observers to remain alert to detect infrequent and unpredictable stimulus events over prolonged periods (6). The inevitable decline of vigilance depends on the frequency and/or speed of signal detections over time. Such decline often occurs from 20 to 30 min after task initiation (15). In some cases, it has been observed as early as the first five minutes (20). Vigilance is required in many occupations, including operation of surveillance systems, quality control inspection in manufacturing, and long-distance driving. Failure to detect and respond to critical signals in these situations could result in disastrous accidents. Exploitation of the appropriate technologies to maintain a high level of vigilance in workers is desirable.

We have previously tried to establish an experi-

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mental method to evaluate the effects of aromatic herbs in relation to a visual discrimination task (19, 27). Aromatic herbs act through olfactory pathways in the brain including the regions of the cerebral cortex, hypothalamus and amygdala, which are different from the visual transduction and processing pathways (26). Pleasant odorants have been reported to improve vigilance performance (10, 16, 30). Our preliminary screening of several essential oils and aromatic herbs suggested that breathing of volatiles emitted from the leaves of *L. noblis* has the potential to improve vigilance performance.

We report here that inhalation of volatiles emitted from the leaves of *L. noblis* improves vigilance performance and the associated temporary changes of heart rate, as investigated by electrocardiogram (ECG). It is known that the subjective effects of odorant also provoke different performance levels; hence, we also evaluated the subjective effects of volatiles emitted from the leaves of *L. noblis* by a visual analogue scale (VAS) against an eight-item questionnaire. The mode of action of the volatile emitted from the leaves of *L. noblis* on vigilance performance was discussed.

MATERIALS AND METHODS

Odorant delivery. The study was carried out using volatiles emitted directly from the leaves of *L. noblis* and delivered with odorless air for 45 min. The leaves of *L. noblis* were cut into 0.5 cm × 0.5 cm pieces. The weight of each sample was 0.1 g (low-dose group) and 3 g (high-dose group), respectively. The odorant delivery system was controlled by a constant-flow olfactometer that forced air at a constant rate of 1.0 L/min through Teflon tubing into a charcoal trap; then the air was put into a 300 mL glass chamber (SANSYO, Japan) with or without the above-mentioned leaves of *L. noblis*, as illustrated in Fig. 1a. Air from the chamber was transmitted through additional tubing to a modified mask immobilized 15 cm from the nose of a participant seated in the experiment room (AVITECS; YAMAHA Corp., Shizuoka, Japan). The experiment room was maintained at 25 ± 2°C during all experiment periods.

Constituent analysis of the volatiles emitted from the leaves of L. noblis. The essential oil of the leaves of *L. noblis* has been known to contain 1,8-cineole, linalool, α-terpinyl acetate and several terpenes (5, 24). The leaves of *L. noblis* were stored at 4°C before performing experiments. The composition of the volatiles emitted from the leaves of *L. noblis* was analyzed by gas chromatography-mass spectrometry (GC-MS) (GC-17A/QP5050; Shimadzu Co., Ltd., Kyoto, Japan), equipped with a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies Inc., Santa Clara, CA, USA) and solid-phase microextraction (SPME) fiber coated with a 65-μm thick layer of polydimethylsiloxane/divinylbenzene (Supelco, Bellefonte, PA, USA). Before use, the fiber was conditioned as recommended by the manufacturer (at 250°C for 30 min). The leaves of *L. noblis* in a sealed 300-mL round-bottom flask were placed at 20 ± 2°C for 30 min. After incubation of the leaves, volatiles emitted from the leaves of *L. noblis* were extracted for 15 min by the fiber, and the fiber was quickly transferred to a GC injector. The temperature program of GC-MS was as follows: 50°C for 2 min followed by increases of 3°C min^{-1} to 150°C, and then increases of 10°C min^{-1} to 200°C, held for 10 min. The other parameters were as follows: injection temperature, 230°C; ion source temperature, 230°C; ionization energy, 70 eV; carrier gas, He at 1.7 mL min^{-1}; mass range, *m/z* 50 to 450. Quantification was obtained from percentage peak areas of the gas chromatogram. Mass spectral database libraries (1) and authentic reference chemicals were used for substance identification. To determine volatile constituents and contents emitted from the leaves of *L. noblis* during the vigilance performance experiment, volatiles emitted from the leaves of *L. nobilis* were collected using a Tedlar PVF bag controlled at a constant-flow rate of 1.0 L min^{-1} for 45 min. Before analysis of the collected volatiles in the bag, 5 μL of phenylethyl alcohol/acetone (concentration at 10 μL/mL) was added as an internal standard and kept there for 30 min. Then, the collected volatiles emitted from the leaves of *L. noblis* were extracted for 15 min by the SPME fiber mentioned above. The fiber was quickly transferred to a GC injector, and the constituents were analyzed as described above.

Participants. The experimental design of the study was approved by Kyushu University and is in accordance with Declaration of Helsinki. Nine healthy male university students (range: 20 to 23 yr) were recruited for the study. All participants met the following criteria: no abnormality of sense of smell, no physical or mental health problems, no drug consumption, and no smoking. The purpose and schedule of the experiments were explained, and signed informed consents were obtained from all participants prior to the study initiation. Before the experiment, participants were instructed to sleep suf-

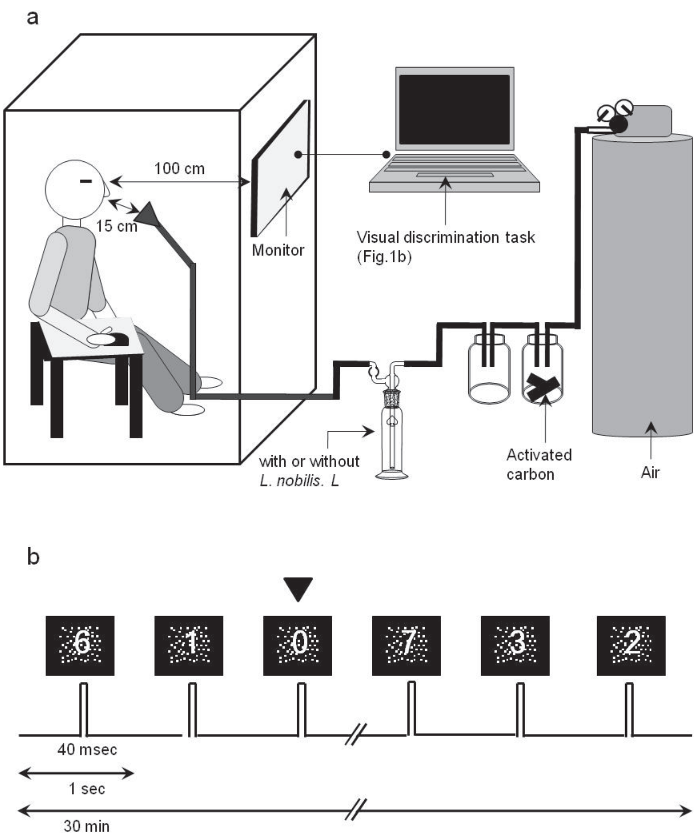

Fig. 1 Schematic diagram of experimental configuration. **a**, Visual discrimination task (VDT) and olfactory system. The odorant delivery system was regulated by a constant-flow olfactometer, which forced air at a constant flow rate of 1.0 L/min. Air from the chamber containing *L. noblis* was transmitted to a modified mask immobilized 15 cm from the nose of the participant. Each participant was seated in front of a VDT monitor at a viewing distance of approximately 100 cm. **b**, VDT program. The program depicted a series of numbers (0–9) with static random dots, and each number was randomly shown for 40 ms. The inter-stimulus interval was 1 s and the task period was 30 min. Participants were instructed to click the PC mouse when the number "0" appeared (black triangle) and not to respond when other numbers appeared.

Table 1 *Experimental procedure*

Period	Time	Actions
Questionnaire period	5 min	Answer to questionnaire
Baseline period	5 min	Keep quiet in sitting position with closed eyes
Working period	30 min	Perform a visual discrimination task
Recovery period	5 min	Keep quiet in sitting position with closed eyes

ficiently, to avoid special activities, such as excessive exercise, one day before the experiment, and not to arrive fatigued or drowsy on the day of the experiment. Consumption of alcohol or medications one day before the experiment, as well as consumption of caffeine drinks on the day of the experiment, was prohibited. Each participant performed the experiment three times, once with no odor, and once each with low-dose and high-dose volatiles from *L. noblis* at intervals of one week.

Visual discrimination task. Participants were tested individually in a 1.3 × 1.8 × 2.0 m industrial acoustics sound room (AVITECS; YAMAHA Corp., Shizuoka, Japan) that was fully sealed from ambient noise and light. During the test, participants were seated in front of a 21.3-inch color LCD monitor (RDT214S, 1600 pixels × 1200 pixels, 300 cd/m^2; Mitsubishi Electric Corp., Tokyo, Japan) controlled by a computer (Windows XP). The view distance was approximately 100 cm (Fig. 1a). The visual discrimination task (VDT) test was previously reported

Table 2 *Constituent analysis of the volatiles emitted from the leaves of L. noblis*

RT (min)	Components	Composition (%)	RT (min)	Components	Composition (%)
6.2	α-thujene	0.2	21.0	α-terpinyl acetate	22.8
6.4	α-pinene	0.8	21.2	eugenol	1.0
7.7	sabinene	5.0	21.5	neryl acetate	0.5
7.9	β-pinene	0.9	21.9	α-copaene	0.6
8.3	myrcene	1.8	22.4	β-elemene	1.0
9.6	1,8-cineole	25.1	22.8	methyl eugenol	1.6
10.6	γ-terpinene	0.3	23.0	α-gurjunene	0.9
11.7	terpinolene	0.2	23.3	*E*-caryophyllene	0.6
12.1	linalool	14.5	23.9	α-guaiene	1.0
14.9	terpinen-4-ol	0.5	25.8	α-humulene	0.4
15.4	α-terpineol	4.7	26.1	α-bulnesene	0.3
17.8	linalyl acetate	0.7	26.6	δ-cadinene	0.3
18.8	bornyl acetate	0.4			
19.9	δ-terpinyl acetate	1.4			

by Matsubara *et al*. (2009) and was 30 min in length. A series of random numbers between 0 and 9 appeared on the screen, and each number was presented for 40 ms/s (Fig. 1b). In the outer frame of the screen at a visual angle of 1.4 × 1.3°, static random noise was presented. The noise was overlaid on each number in order to increase the difficulty of the task. The series of numbers was presented at a 0.91 × 0.68° view angle in diameter. Participants were asked to click a mouse when the number "0" appeared but not to click the mouse when other numbers appeared. Two indices of work performance were calculated by the software: 1) the rate of true hits, p(tH), which was the rate of response to correct targets minus the rate of response to incorrect targets; and 2) the reaction time which was the time taken to click when the correct targets appeared. The experimental procedure is described in Table 1. Each experiment was performed by the same time schedule. Air with or without volatiles emitted from the leaves of *L. noblis* was given from the questionnaire period to the recovery period.

ECG recording. To evaluate cardiac autonomic nervous activity, we used a power spectral analysis of R-R intervals. Power spectral analyses were performed using the MemCalc system (MemCalc Ver. 2.5; Suwa Trust Co., Tokyo, Japan). Heart rate variability (HRV) was assessed by a two-dimensional Lorenz scatter plot of successive intervals versus the immediately preceding R-R interval (29). 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.

Subjective effects of the volatiles emitted from the leaves of L. noblis. The subjective effects of the volatiles emitted from the leaves of *L. noblis* were measured before performing a visual discrimination task. As the result of our preliminary screening of several words to explain their subjective effects, eight words were selected. A visual analogue scale (VAS) was used as a measurement instrument by indicating a position along a continuous line between two end-points. These consisted of an eight-item questionnaire designed to differentiate subjective responses to different concentrations of the odorant: "fresh-stale," "like-dislike," "calm-stormy," "pleasant-unpleasant," "safety-danger," "acrid-mild," "active-inactive," and "cloudy-clear."

Statistical analysis. Several statistical tests were performed to reveal the changes in the parameter values between the experiment groups. Statistical analysis was performed using SPSS 15.0 J for Windows (SPSS Japan Inc., an IBM Company, Tokyo, Japan). The difference of the values at certain time points between the *L. noblis* group and the control group was analyzed by the Student's *t* test. For the subjective effects, one-way ANOVA with Tukey post hoc test was used to evaluate the differences between each group. The values are expressed as means ± SEM. P values < 0.05 were considered significant and values < 0.1 as indicating a tendency.

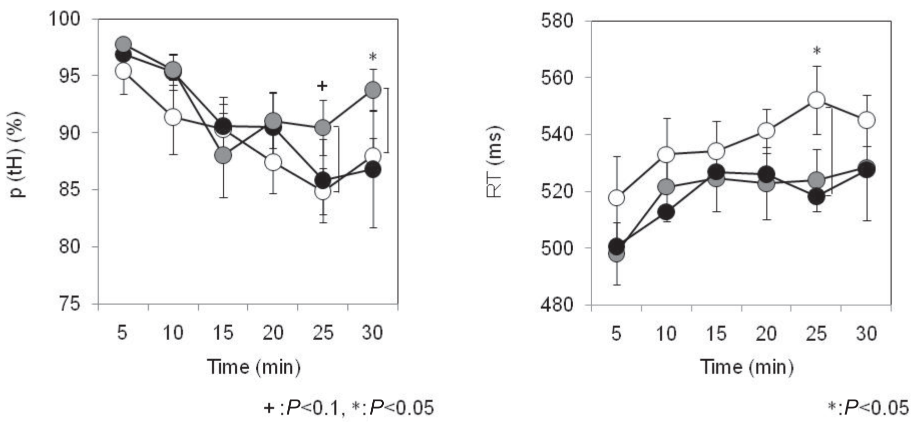

Fig. 2 Time course of vigilance performance in visual discrimination task. The participants were instructed to click the PC mouse when the number "0" appeared and not to respond when other numbers appeared. The program provided two indicators reflecting work performance: [1] the rate of true hits, p(tH), indicating the rate of response to correct targets except for the rate of response to error targets, and [2] reaction time, RT, indicating the time taken to click when the correct targets appeared. Circles represent p(tH) and RT values in low-dose *L. noblis* (gray), high-dose *L. noblis* (black) and control groups (white). Compared with the control, p(tH) in low-dose *L. noblis* group increased with tendency at 25 min and significance at 30 min ($P < 0.1$ at 25 min, $P < 0.05$ at 30 min). RT in high-dose *L. noblis* group significantly decreased at 25 min ($P < 0.05$). Asterisks indicate statistical significance (*$P < 0.05$). + indicates statistical tendency ($P < 0.1$). Data are shown as means ± SEM.

RESULTS

We investigated the effects of the volatiles emitted from the leaves of *L. noblis* at two different doses (0.1 g for low-dose and 3.0 g for high-dose) on vigilance performance in a visual discrimination task. ECG and subjective effects were recorded and analyzed in our experimental system (Fig. 1a, Table 1). The volatile constituents emitted from the leaves of *L. noblis* were analyzed by GC-MS.

Constituent analysis of the volatiles emitted from the leaves of L. noblis

The chemical constituents of the volatiles emitted from the leaves of *L. noblis* were analyzed by GC-MS. Twenty-six constituents were detected; the main ones were 1,8-cineole (25.1%), linalool (14.5%), α-terpinyl acetate (22.8%), sabinene (5.0%), and α-terpineol (4.7%). Monoterpenes and sesquiterpenes comprised the majority of the *L. noblis* leaves (87.5%) (Table 2). There were seven constituents that each participant inhaled during the experiment; α-pinene (0.3 μg for low-dose group and 1.5 μg for high-dose group, respectively during 45 min), sabinene (1.4 μg and 13.8 μg), β-pinene (0.2 μg and 1.7 μg), 1,8-cineole (8.9 μg and 102.3 μg), linalool (5.2 μg and 81.0 μg), α-terpineol (1.3 μg and 21.4 μg), α-terpinyl acetate (6.1 μg and 71.2 μg). The total amount of volatiles in the 45 L of air breathed in the experimental period (from the questionnaire period to the recovery period) was 23.4 μg in the low-dose group and 292.9 μg in high-dose group, respectively.

Visual discrimination task

The level of vigilance performance was determined by the rate of true hits—p(tH)— and reaction time. The values of p(tH) and reaction time were defined as the average of each five-minute period (Fig. 2). From 5 min to 20 min, p(tH)s were no different between the high or low-dose of *L. noblis* groups and the control group. At 25 min, p(tH) was 90.5 ± 2.4 (mean ± SEM) for the low-dose *L. noblis* group and 84.9 ± 2.0 for the control group. At 30 min, p(tH) was 93.8 ± 1.8 for the low-dose *L. noblis* group and 88.0 ± 1.6 for the control group. The difference in p(tH) between the control and low-dose group indicated tendency and significance ($P < 0.1$ at 25 min, $P < 0.05$ at 30 min). This result showed the decline of vigilance performance was inhibited by breathing low-dose *L. noblis*. Also the reaction times in high- and low-dose *L. noblis* groups during all periods were almost same and slightly smaller than those in the control group (for the high dose group at 25 min, $P < 0.05$).

Analysis of heat rate variability

HRV obtained by intervals between successive R-waves of ECG (R-R intervals) exhibited prominent changes during the performance of the visual discrimination task. Lorenz plots of R-R intervals for every five minutes were plotted. Mean R-R in-

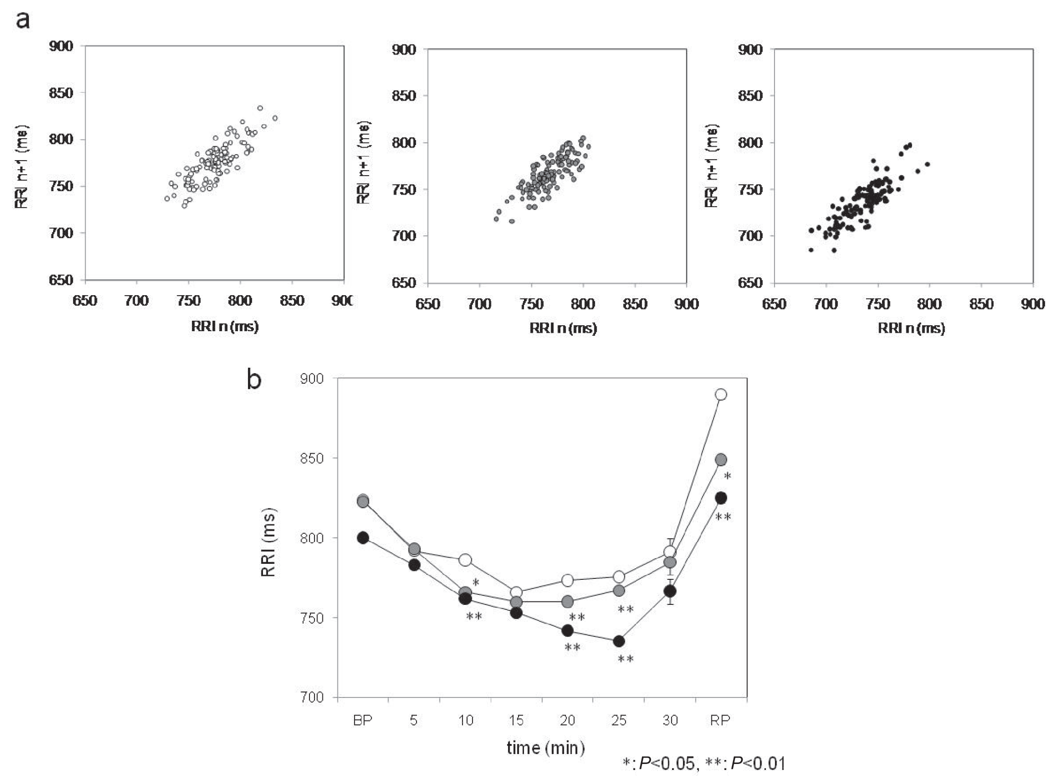

Fig. 3 Differences in R-R intervals. **a**, Lorenz plots of R-R intervals. The heart rate variability (HRV) for a 5-min baseline period (BP), 30-min working period divided into 5-min segments and 5-min recovery period (RP) were plotted. RRI_n and RRI_{n+1} indicate the N^{th} and $(N + 1)^{th}$ R-R interval, respectively. Circles represent R-R intervals in low-dose *L. noblis* (gray), high-dose *L. noblis* (black) and control groups (white). **b**, Time course of mean R-R intervals. Circles represent the mean R-R intervals in low-dose *L. noblis* (gray), high-dose *L. noblis* (black) and control groups (white). Compared with the control, the mean R-R intervals were significantly lower in the low-dose *L. noblis* group ($P < 0.05$ at 10 min, $P < 0.01$ at 20 min, $P < 0.01$ at 25 min, $P < 0.05$ at RP) and the high-dose *L. noblis* group ($P < 0.01$ at 10 min, $P < 0.01$ at 20 min, $P < 0.01$ at 25 min, $P < 0.01$ at RP). Asterisks indicate statistical significance ($^{**}P < 0.01$, $^{*}P < 0.05$).

tervals, defined as the averages of five-minute periods, were used for statistical analysis. Statistical dispersion patterns and shapes of the plots of R-R intervals were not different between the *L. noblis* groups and the control group (Fig. 3a). At 10 min, mean R-R intervals (± SEM) showed a significant difference between the *L. noblis* groups and the control group (786.2 ± 1.7) (766 ± 1.4; low-dose, $P < 0.05$, 761.9 ± 1.7; high-dose, $P < 0.01$). At 20 min, mean R-R intervals showed a significant difference between the *L. noblis* groups and the control group (773.5 ± 1.8) (760.1 ± 1.7; low-dose, $P < 0.01$, 742 ± 1.8; high-dose, $P < 0.01$). At 25 min, mean R-R intervals showed significant differences between the *L. noblis* groups and the control group (775.7 ± 1.9) (767.2 ± 1.8; low-dose, $P < 0.01$, 735.4 ± 2; high-dose, $P < 0.01$). During the recovery period, mean R-R intervals showed significant differences between the *L. noblis* groups and the control group (890.1 ± 2.8) (849 ± 3.2; low-dose, $P < 0.05$, 825.1 ± 3; high-dose, $P < 0.01$). The decrease of R-R intervals shows that the heart rate is faster. Our results suggest that breathing the volatile constituents of the leaves of *L. noblis* stimulates the autonomic nerve, which relates to the adjustment of heart rate for visual discrimination task performance.

High-frequency components and the ratio of low frequency to high frequency of HRV

Compared with the control, smaller high-frequency components (HF, 0.15–0.4 Hz) were obtained in the baseline period and at 25 min during the working period in the high-dose *L. noblis* group with tendency. The levels of HF components and ratios of LF/HF were defined by the average of every five-minute period (Fig. 4). The HF components were 317.1 ± 65.2 (ms^2) for the high-dose *L. noblis* group and 437.1 ± 90.8 (ms^2) for the control group during the baseline period before performing the work. These values were 331.0 ± 86.1 (ms^2) for the high-dose *L.*

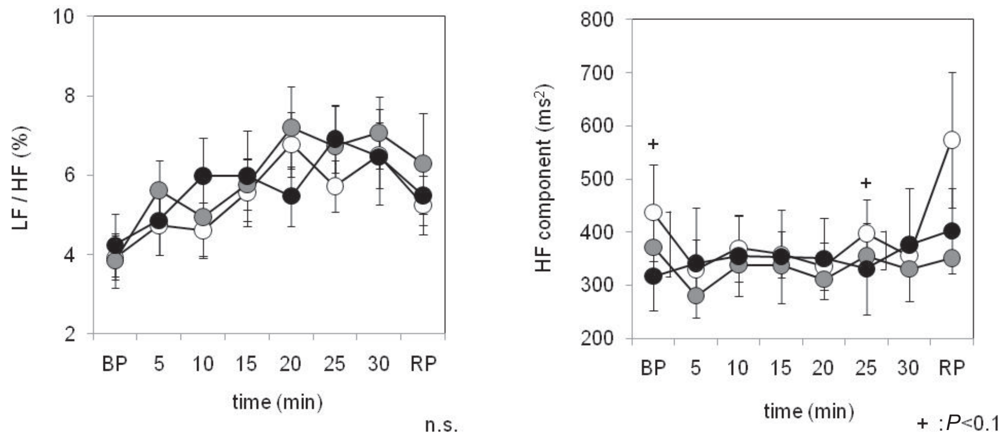

Fig. 4 HF components and LF/HF ratios during all periods. Spectral analysis of two major components, the ratio of low frequency to high frequency (LF/HF, 0.04–0.15 Hz/0.15–0.4 Hz) and high-frequency components (HF, 0.15–0.4 Hz) are shown. BP, 5-min baseline period; 30-min working period divided into 5-min segments; RP, 5-min recovery period. Circles represent LF/HF ratios and HF components in the low-dose *L. noblis* (gray), high-dose *L. noblis* (black) and control groups (white). Compared with the control group, the HF components in the high-dose *L. noblis* group were lower in the BP ($P < 0.1$) and at 25 min in the WP ($P < 0.1$) with tendency. + indicate statistical tendency ($P < 0.1$). Data are shown as means ± SEM.

noblis group and 398.0 ± 64.0 (ms^2) for the control group at 25 min during the working period ($P < 0.1$ at the baseline period, $P < 0.1$ at 25 min). The LF/HF ratios for 20–30 min were 7.0 ± 0.2 (%) for the low-dose *L. noblis* group, slightly higher than those of control group, at 6.3 ± 0.5 (%) ($P = 0.12$). The HF components and the LF/HF ratios of HRV reflect cardiovascular autonomic nerve activities. These findings suggest that the breathing of volatile constituents of the leaves of *L. noblis* stimulates the heart rate for visual discrimination task performance.

Subjective effects of the volatiles emitted from the leaves of L. noblis

The level of subjective effects of *L. noblis* 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 word descriptors at each end. The participants marked a point on the line indicating their subjective response to the aroma that they had been breathing. The VAS score was determined by measuring in millimeters from the left-hand end of the line to the point that participants marked. The VAS scores showed no differences between the low-dose *L. noblis* and control groups. On the other hand, significant differences appeared between the scores of the high-dose of *L. noblis* group and low-dose or control group (Fig. 5). The scores of "calm-stormy" were 46.6 for the high-dose, 70.0 for the low-dose and 65.0 for the control group (low-dose, $P < 0.05$, control, $P < 0.1$). The scores of "pleasant-unpleasant" were 55.4 for the high-dose and 74.2 for the low-dose group ($P < 0.1$). The scores of "safety-danger" were 44.8 for the high-dose and 62.7 for the control group ($P < 0.05$). The scores of "acrid-mild" were 70.1 for the high-dose, 47.0 for the low-dose and 46.4 for the control group (low-dose, $P < 0.1$, control, $P < 0.1$). Finally, the scores of "cloudy-clear" were 19.2 for the high-dose group, 42.9 for the low-dose and 58.4 for the control group (low-dose, $P < 0.1$, control, $P < 0.01$).

DISCUSSION

Decline in vigilance depends on the frequency and/or speed of signal detections over time (15). To prevent disastrous accidents and economic loss, a high level of vigilance is required in many jobs. Decrease of vigilance has a robust relationship with elevated sleepiness and fatigue (7). Technologies to detect lowered states of arousal have been improved (8, 22). In these technologies, the sensitivity of detection may become an important factor because there are limited ways to detect the accumulation of sleepiness and fatigue. Such sensitivity is strictly required for the prevention of serious accidents. Several studies have focused on the reduction of fatigue resulting from a task requiring sustained attention using aromatic herbs. They have suggested that aromatic herbs are useful in improving vigilance during task performance (13, 27, 30). In this study, we observed tendency (at 25 min) and significance (at 30 min) of higher rates of true hits in the low-dose *L. noblis* group (Fig. 2), suggesting that the low-

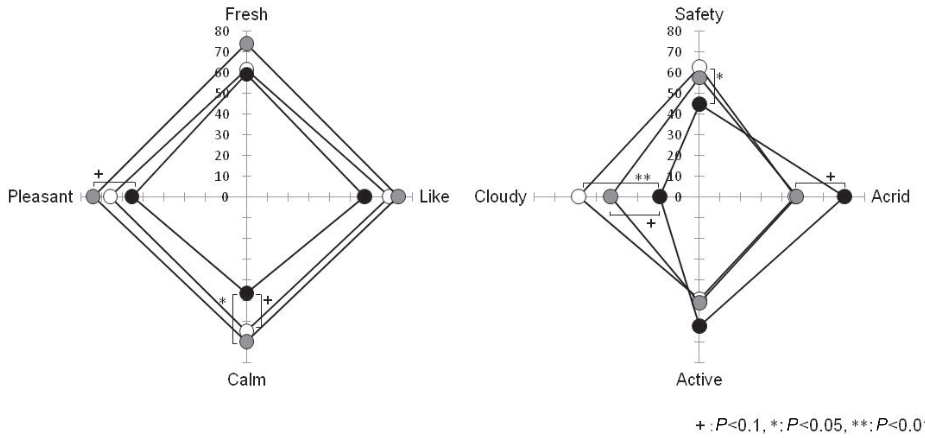

Fig. 5 Subjective effects of the volatiles emitted from the leaves of *L. noblis*. The visual analogue scale (VAS) scores for an eight-item questionnaire are shown; "fresh-stale," "like-dislike," "calm-stormy," "pleasant-unpleasant," "safety-danger," "acrid-mild," "active-inactive" and "cloudy-clear". Circles represented the VAS scores in the low-dose *L. noblis* (gray), high-dose *L. noblis* (black) and control groups (white). Compared with the high-dose *L. noblis* group and low-dose or control groups, the scores of "calm-stormy" (low-dose, $P < 0.05$, control, $P < 0.1$), "safety-danger" (control, $P < 0.05$), "acrid-mild" (low-dose, $P < 0.1$, control, $P < 0.1$) and "cloudy-clear" (low-dose, $P < 0.1$, control, $P < 0.01$).

dose volatiles prevented the decrease of p(tH) found in controls from 20 to 30 min after the initiation of a task. This is the first report that volatiles emitted from the leaves of *L. noblis* have the potential to prevent the decrease of vigilance performance in a visual discrimination task. Our result is partly consistent with a previous report that the percentages of correct detections were higher while subjects breathed of muguet and peppermint fragrances (30). The amounts of aromatic herbs influence the pharmacological and psychological responses; high doses sometimes induce the subject's discomfort. The reaction times in high- and low-dose *L. noblis* groups during all periods were slightly smaller than those in the control group. In this study, we observed that the expected decrease of vigilance, especially p(tH)s was inhibited by low-dose *L. noblis* but not by high-dose *L. noblis*, suggesting that the pharmacological and psychological activations during task performance were probably different in the two groups toward p(tH)s. To explain the results, a further experiment was performed as described below.

Cardiovascular function is controlled by the autonomic nervous system, composed of sympathetic and parasympathetic nervous systems. Previous reports have indicated that several aromatic herbs increase cardiovascular autonomic reactivity by breathing (11, 28). Lorenz scatter plots of R-R intervals, which provide beat-to-beat dynamic measure of HRV, have distinctive and characteristic patterns (29). Low-frequency (LF, 0.04–0.15 Hz) and high-frequency (HF, 0.15–0.4 Hz) components of HRV are used to assess the degree of inputs to the heart from the autonomic nerves (18, 25): HF components and LF/HF ratios reflect sympathetic and parasympathetic nerve activities, respectively. Thus, the analyses of R-R intervals, HF components and LF/HF ratios directly indicate cardiovascular function. There is no significant difference of the value of LF/HF ratios and HF components during all period among three groups (Fig. 4). However, in comparison with the control group, we obtained slightly higher LF/HF ratios for 20–30 min in the low-dose *L. noblis* group and smaller HF components in the baseline period and at 25 min during the working period in the high-dose *L. noblis* group with tendency. On the other hands, we observed decreases of the mean R-R interval with significance in both the high- and low-dose *L. noblis* groups during task performance (Figs. 3, 4), suggesting that the sympathetic nerve is activated and cardiovascular function is elicited by breathing the volatiles. This is the first observation of the effect of *L. noblis* and shows the possibility that it elevates cardiovascular function. Previous reports have indicated that vigilance was correlated significantly with subjective perceived fatigue or sleepiness and cardiovascular function (3, 4, 23). In these reports, the activation of cardiovascular parameters was shown differently, which should depend on task difficulty and workload. Our result is partly consistent with a previous report that the increase of R-R intervals and response time shows the decrease of vigilance (23), suggesting that the analysis of R-R intervals is useful for estimating vigilance performance levels. Our results also showed that the breathing of volatiles emitted from leaves of *L. noblis* enhances this arousal level. However, we could not explain why low-dose *L. noblis* improved vigilance, especially p(tH)s in the visual discrimination task and the high dose did not.

Pleasant and unpleasant odorants have known to provoke different degrees of vigilance. Previous reports have indicated that the pleasant odorants improve visual vigilance in a sustained visual discrimination task (10, 16, 30). The subjective effects of *L. noblis* in high- and low-dose groups are significantly different in some parameters (Fig. 5). In this study, the VAS scores were constructed by the combination of positive and negative words. The perceptions of "stormy," "danger" and "acrid" are closely related to negative emotion. Our results showed that the scores of these negative words were higher for the high-dose group than low-dose or control groups. More specifically, the scores of "pleasant" were lower in the high-dose group. Considering together with the results of Fig. 2, which indicated the low-dose *L. noblis* group but not the high dose *L. noblis* group have higher p(tH)s at 25 and 30 min than the control group, the perception of pleasant and relaxing odorants could improve vigilance performance, such as p(tH)s. Our results may be linked to the lack of effect of high-dose *L. noblis* on vigilance scores such as p(tH)s, at least in part.

In this study, we identified the volatiles emitted from the leaves of *L. noblis*; among them, α-pinene, β-pinene, sabinene, 1,8-cineole, linalool, α-terpineol, α-terpinyl acetate and 1,8-cineole were the highest. Our findings suggested that the volatiles emitted from the leaves of *L. noblis* enhanced vigilance and therefore prevented the decrease of vigilance found in controls. Using an adequately low dose of the volatiles seems important for this activity, especially p(tH)s. Previous reports have suggested that 1,8-cineole improves vigilance performance in comparison to linalool (12, 13). However, the contribution of each volatile to this activity and the effect of their combination and optimal concentrations could not be discerned from our study design. Further experiments using each volatile in our assay system is needed for clarification of their mode of actions.

In the present study, the value of p(tH) from 20 to 30 min in a visual discrimination task was higher in the only low-dose *L. noblis* group than in the control group. These findings indicate that inhalation of a low concentration of volatiles emitted from the leaves of *L. noblis* may maintain vigilance performance by facilitating cardiovascular function without inducing detrimental emotional effects.

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