1. INTRODUCTION

There are a variety of teas in the world, and their favorable effects have been investigated for a long time. With the increasing indications of favorable health effects of green tea components, e.g., catechin, theanine and caffeine, green tea has come to be widely drunk in many parts of the world. Although some kinds of tea with especially strong flavor, e.g., black tea and herbal tea, have been studied with respect to the physical and psychological benefits of their odor, few studies have examined the benefit of smelling green tea.

Some odor components of green tea have been shown to have an anti-stress effect. For instance, a study on the effects of green odor, that is, (E)-2-hexenal, (Z)-3-hexenol, one of the main odor components of green tea, on the brain have shown that green odor components stimulated the release of dopamine, which regulates brain functions, mood and attention [1].

In a recent paper, Watanabe et al. (2011) [2] indicated that the inhalation of green odor can alleviate or prevent a depressive-like state in rats. In a human study, green odor also induced changes of event-related potential P-300 and perceived pleasure [3]. Linalool, one of the major odor components of green tea, has been shown in experimental mouse anxiety models to be useful as a means to attain relaxation and counteract anxiety [4, 5], and in a human experiment it reversed the physiological alteration parameters produced by stress [6]. The odor of green tea consists not only of these components but also other components that can have an anti-stress effect. Although the effect of a particular odor component of green tea has been reported, the effect of green tea odor as a whole has not been studied.

The purpose of this study was to investigate the electroencephalographic activity after smelling two kinds of green tea and to examine any anti-stress effects of smelling green tea. Ten subjects (6 male and 4 female) participated in this study. We used two kinds of green tea, Sagara and Shaded White Tea, which were made from the same kind of tea leaf but by different manufacturing processes. We used an arithmetic task and an auditory discrimination task to test mental stress. An electroencephalogram (EEG) was recorded before and after smelling the tea samples and before and after performing the mental tasks. Subjective ratings about mental status were obtained with the profiles of mood states (POMS) and the visual analog scale (VAS) at the end of each task. EEG was recorded from 4 scalp positions of the international 10/20 system (F3, F4, O1 and O2), and EEG activity was estimated for 4 frequency bands (alpha1, alpha2, beta1 and beta2). The results showed that the subjective rating about relaxed feeling increased and electroencephalographic activities changed after smelling green tea, especially Shaded White Tea, suggesting that smelling green tea may have anti-stress effects.

Keywords: Green tea, odor, EEG (Electroencephalogram), mental stress

2. MATERIALS AND METHOD

The experiment conducted in this study was approved by the research ethics committee of the University of Shizuoka and was carried out in accordance with the Declaration of Helsinki.

2.1 Subjects

Ten subjects (6 male and 4 female) between 21 and 24 years of age (M=23.2, SD=1.23) participated in this experiment. All subjects were healthy volunteers and right-handed, and gave written informed consent for study participation prior to their participation.

2.2 Odor administration

We used two kinds of green tea, Sagara and Shaded White Tea, and water as a control. Sagara is a green steam tea made by the Japanese Yabukita tea plant. Shaded White Tea is also made tea this plant, but it is made by a
different manufacturing process. Shaded White Tea is made with perfectly blocked sunlight before plucking. One of the main biochemical pathways of a plant’s odor component is the chloroplasts. Therefore, the odor of Shaded White Tea was different from that of Sagara. Odor samples were extracted in paper cups with hot water from 5 g of each type of tea leaf. To modulate the intensity of the sample odors, we adjusted the hot water temperature to 98 degrees C, and the liquid measure to 90 ml for Sagara and 60 ml for Shaded White Tea and water. After extraction, we covered the cup with aluminum foil until there was a direction for the subjects to smell the odor from the cup. The subjects smelled the odor by holding the cup under their nose for 1 minute.

2.3 Stress load task
An arithmetic mental task (AT) and an auditory oddball target detection task (DT) were imposed as mental stress tasks.

In the AT, subjects were requested to add two numbers randomly displayed on a PC monitor and type the answer in the answer column with a numerical keypad as quickly and accurately as possible. The AT was a 10-minute set that was repeated 4 times.

In the DT, subjects responded to target stimuli that occurred infrequently and irregularly within a series of standard stimuli [7]. In this study, repetitions of a high-pitched tone, 2000 Hz, lasting for 0.1 second, were the target stimuli, and repetitions of a low-pitched tone, 1000 Hz, lasting for 0.1 second, were the standard stimuli. Subjects were requested to click the left mouse button as quickly as possible when they heard the target stimuli. The DT was a 5-minute set that was repeated 3 times.

2.4 EEG recording
EEG was recorded from 4 scalp positions of the international 10/20 system (F3, F4, O1 and O2). Electrooculogram (EOG) was recorded at the left eye. EEG and EOG data were amplified and A/D converted by a versatile amplification unit (polymate AP1132, TEAC Corporation). High- and low-pass filters were set to 0.05 Hz and 0.16 Hz, and 60 Hz and 15 Hz for EEG and EOG, respectively. The sampling rate was 1000 Hz. EEG activity was analyzed with fast Fourier transform (Vital Tracer and BIMITUS, KISSEI COMTEC CO. LTD.) for 4 frequency bands: 8–10 Hz (alpha 1), 10–13 Hz (alpha 2), 13–20 Hz (beta 1) and 20–30 Hz (beta 2).

2.5 Subjective assessment
In this experiment, the short profile of mood states (POMS) and the visual analog scales (VAS) were used for subjective assessment of mood status. Subjects completed questionnaires while resting, after smelling the samples and after performing the mental tasks.

The POMS consists of six items: Tension-Anxiety (T-A), Depression-Dejection (D), Anger-Hostility (A-H), Vigour-Activity (V), Fatigue-Inertia (F) and Confusion-Bewilderment (C). Participants are asked to rate how each item applies to their mood state (past or current) on a 5-point Likert-type scale, ranging from 0 (Not at all) to 4 (Extremely). Scores for the one positive item (V) are reversed, and the total mood disturbance (TMD) rating is obtained by calculating the sum of the scores. TMD change scores were calculated as described in the Design section. Since a higher TMD score indicates a more negative affective state, thus positive changes in mood are reflected by negative changes to TMD scores [8].

The VAS used in this experiment was a 10-cm line, with the end point 0 for “not feel” and 10 for “strongly feel.” Subjects were asked to make a mark on the line that represented their mood at the time. The VAS included some questions about the subject’s feelings with regard to pressure, drowsiness, stress, relaxation, fatigue, security and tension, and about preference with regard to the odor of samples.

2.6 Procedure
Figure 1 shows the procedure of the experiment. The experiments were carried out in a quiet room with the
temperature of about 25 degrees C and the relative humidity of about 50%. Subjects entered the room and sat in front of a PC. After electrodes were attached, EEG was measured for 1 minute and subjects completed questionnaires for subjective assessment of their mood state during rest. Next, subjects performed the DT (5 min) under normal status of not having smelled either test odor. Then, the subjects smelled the samples while EEG was measured again. They also completed questionnaires again. After subjective assessment, subjects performed “task1”, i.e., 2 trials of the AT (10 min×2) and 1 trial of the DT (5 min). Between tasks, subjects smelled the samples (1 min). After subjects completed task1, EEG measurements and subjective assessment were conducted. In “task2”, measurement and assessment were conducted again as in “task1”.

2.7 Statistic analysis
All analyses were performed with IBM SPSS Statistics version 19. ANOVA procedures carried out using the Nonparametric Friedman test or Kruskal-Wallis test. The Wilcoxon signed-rank tests or Mann-Whitney’s U-test was then performed as a multivariate ANOVA (MANOVA) procedure. The significance level was set at p<0.05.

3. RESULTS

3.1 EEG data
Figure 2 shows alpha band (alpha1 and alpha2) power at the frontal region (F3 and F4) when subjects were smelling one of the test odors. When subjects smelled Shaded White Tea, alpha2 activity in the left frontal area (F3) increased significantly (p<0.05, vs. smelling water).

Figure 3 shows beta band (beta1 and beta2) power at the occipital region (O1 and O2) when subjects were smelling one of the test odors. When subjects smelled Shaded White Tea, beta2 activity in the left occipital area (O1) increased significantly (p<0.05, vs. smelling water).
the occipital region (O1 and O2) when subjects were smelling one of the test odors. When subjects smelled Shaded White Tea, beta1 activity in the left and right occipital area (O1 and O2) increased significantly (p<0.05, vs. smelling water), and Beta2 activity also increased significantly in the left occipital area (O1) (p<0.05, vs. smelling water) and in the right occipital area (O2) (p<0.1, vs. smelling water).

3.2 Task performance

Figure 4 shows the percentage of AT correct answers. In the first 10 min of the AT, the percentage of correct answers was slightly higher after smelling Shaded White Tea (p<0.1, vs. after smelling water).

Figure 5 shows the change of DT response time from the first DT to the second or the third DT. After the third DT, the change of response time was significantly faster after smelling Shaded White Tea (p<0.05, vs. smelling water).

3.3 Subjective assessment

Figure 6 shows the Vigor-Activity (V) score in POMS. After task1, the V score was slightly higher after smelling Shaded White Tea (p<0.1, vs. smelling water). Also after task2, the V score was significantly higher (p<0.05, vs. smelling water) or slightly higher (p<0.1, vs. smelling Sagara).

Figure 7 shows Relaxing score in the VAS. After smelling Sagara or Shaded White Tea, the Relaxing score was
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significantly higher (p<0.05, vs. smelling water) during the time of smelling the test odor, and the higher scores continued throughout the performance of task1 (p<0.05, vs. smelling water). For task2, the Relaxing score was slightly higher after smelling Shaded White Tea (p<0.1, vs. smelling water).

4. DISCUSSION

As shown in the previous section, when smelling Shaded White Tea, the band power of alpha2 and beta in the frontal and occipital regions increased.

The upper alpha band, i.e., the alpha2 band, has been considered to be specifically related to semantic information processing [9], and also to good cognitive performance in the time interval preceding a task [10]. The beta band is suggested to play an important role in attention or higher cognitive functions [11] and to be related to good performance [12]. Gross et al. (2004) indicated that changes in synchronization in the beta band reflect changes in the attentional demands of the task and are directly related to behavioral performance [13]. With these findings in mind, we noted that the task performance in our study was also improved with increased band power of alpha2 and beta by smelling Shaded White Tea in the time interval preceding a task. Our results thus
agree with those of previous studies and suggest that smelling Shaded White Tea might have the effect of increasing the band power of alpha2 and beta, therefore improving task performance.

Eoh et al. (2005) and Jap et al. (2009) found changes of EEG by driver’s fatigue and suggested that EEG activity was lowered by fatigue [14, 15]. The task performance of the OT became worse in the present study under the condition of smelling water. This might have caused an increased feeling of fatigue and decreased vigor level in response to the time-consuming cognitive tasks. On the other hand, under the condition of smelling Shaded White Tea, the percentage of AT correct answer and the OT response were better than those under the condition of smelling water. These results also indicated that the odor of Shaded White Tea had the effect of reducing the depression of EEG activity caused by fatigue and improving task performance.

Compared with the result of smelling water, a higher (V) score in POMS with the smelling of Shaded White Tea was maintained after task1 and task2, while the Relaxing scores in VAS with the smelling of Sagara and Shaded White Tea were higher until task1 and until task2, respectively. Consequently, the odor of Shaded White Tea may induce positive emotions such as vigor and a sense of relaxation, and these differences of mood status caused by smelling this odor may have affected the EEG activity and task performances. However, Sagara didn’t show a significant effect on EEG activities, nor on task performance. That is to say, Shaded White Tea might have a larger anti-stress effect physiologically and psychologically, whereas Sagara only showed a psychological effect in this study.

5. CONCLUSION

In this study, the anti-stress effect of smelling green tea was investigated using EEG, subjective assessment and task performance.

The results are summarized as follows:
1. The band power of alpha and beta in the frontal and occipital regions increased with smelling Shaded White Tea.
2. The percentage of AT correct answers and the DT response time were improved with smelling Shaded White Tea.
3. The (V) score in POMS with smelling Shaded White Tea was maintained after task1 and 2, and the Relaxing scores in VAS with the smelling of Sagara and Shaded White Tea were higher until task1 and until task2, respectively.

These results suggest that the odor of Shaded White Tea may induce a positive emotion such as vigor and or a sense of relaxation, and may affect EEG activity and task performance.

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