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ORIGINAL ARTICLE

Effect of Smelling Green Tea Rich in Aroma Components on EEG Activity and Memory Task Performance

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Abstract: We investigated the memory task performance and the central nervous activity after smelling two kinds of pan-fired Japanese green tea to examine their physical and psychological effects. Twenty eight subjects participated in this study. We used Koushun and Kouju for test samples, which were made by different manufacturing processes. After smelling each odor sample, a memory task and an arithmetic task were used to test mental stress. Electroencephalogram (EEG) was recorded before and after smelling the test samples, and EEG activity was estimated for 4 frequency bands (alpha 1, alpha 2, beta 1 and beta 2). The profiles of mood states (POMS) and the visual analog scale (VAS) after mental stress task were completed for subjective assessments. The results showed that the odor of Kouju may induce a positive emotion. It may also affect the EEG band power of beta 1 at right frontal region and improve memory task performance.

Keywords: *electroencephalogram (EEG), memory task, green tea*

1. INTRODUCTION

A growing literature demonstrates several health benefits of green tea based on its chemical composition, including prevention and/or control of different types of cancer, heart disease, liver disease, atherosclerosis, hypertension, diabetes, metabolic syndrome and obesity, as well as antibacterial, antiviral and antifungal activities [1-4]. Some odor components of green tea, such as green odor and linalool, have also been shown to have an anti-stress effect in animal and human studies [5-10]. In our previous paper, smelling green tea showed an increase of the subjective rating about relaxed feeling and a less decrease in vigor score after stress loads, and electroencephalographic (EEG) activities changed after smelling green tea, suggesting that smelling green tea may have anti-stress effects. The more, different types of green tea, depending on how they were processed, not only showed their different effects on the mood, but also on EEG activity and task performance [11]. We found that smelling the Shaded white tea induced a more positive emotion, and affected EEG activity and task performance more than the normal green tea did.

Meanwhile, a growing attention has been paid to the pan-fired green tea by its rich flavor. It does not undergo the usual steam treatments of usual Japanese tea but processed by roast and roll method. This process develops flower-like sweet, mildly roasted flavors. Using different cultivar and/or manufacturing by different process can produce a large variety of its aromatic character. We hypothesize that smelling different kinds of this richly flavored green tea might affect people in different way physically and psychologically depending on their special odor characters. However, to our knowledge, no other research has been conducted on the influence of smelling different kinds of pan-fired Japanese green tea.

In this study, we assessed the anti-stress effects of two kinds of pan-fired green tea, i.e. Koushun and Kouju, together with the warm water as a control sample, on central nervous activities in healthy people by measuring EEG and mental task performance. We also evaluated subjects' Profile of Mood States (POMS) [12] scores and the Visual Analogue Scales (VAS) scores as subjective ratings on mental state.

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.

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2.1 Participants

Twenty-eight healthy and right-handed volunteers (14 males, 14 females, ages: 22.5 ± 1.5 years old) participated in the experiment individually. All participants were requested to avoid eating or drinking except for water intake beginning 3 hours before the start of the experiment.

2.2 Treatment

Koushun and Kouju, the tea leaves used as test samples were manufactured from a collaboration of Shizuoka Tea Research Center and Honyama research group. The concentrations of caffeine in the two samples were analyzed by HPLC (High Performance Liquid Chromatography) with UV detection at 280nm, and the samples' odor components were analyzed by FEDHS (Full Evaporation Dynamic Head Space) method. The results are shown in Table 1. Koushun contains more floral components than Kouju, such as Methyl jasmonate and indole, thus Koushun smells like flowers. Kouju contains more aromatic odor of Benzyl nitrile, and it smells like fruits such as grapes or cantaloupe melon.

Table 1: Concentration of caffeine and odor components in tea samples (peak area divided by internal standard)

Components	Koushun	Kouju
caffeine	0.82	1.11
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.45	0.83
1-Dodecanol	1.42	1.38
1-Hexanol, 2-ethyl-	0.79	1.06
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	1.38	1.13
2(3H)-Furanone, 5-ethyldihydro-	6.36	5.18
2H-1-Benzopyran-2-one	6.28	5.53
2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)-	24.99	22.46
2-Propanol, 1-(2-methoxypropoxy)-	1.46	1.08
Benzoic acid, 2-ethylhexyl ester	0.89	1.09
Benzyl Alcohol	1.36	1.68
Benzyl nitrile	1.82	5.03
Butanoic acid, 3-hexenyl ester, (Z)-	2.33	1.71
Dimethyl Sulfoxide	0.89	0.69
Ethanol, 2-(2-ethoxyethoxy)-	1.43	1.14
Indole	1.53	1.29
Methyl jasmonate	3.89	2.42
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1.13	1.09
Phenol	0.99	1.00
Phenylethyl Alcohol	3.76	3.34
Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	1.09	1.22
Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	1.08	1.16

* odor components were estimated from MS data

Odor samples were extracted in paper cups with 50 ml of hot water at 70 degrees C from 3 g of each type of tea leaf or without tea leaf for totally 3 test samples, i.e. Koushun, Kouju and warm water. Then, we covered the cup with a silicon cover for about 2 min until it was served to the subjects under their nose and let the subjects smell the odor from the cup for 1 minute with their eyes closed.

2.3 Stress load mental tasks

A memory task and an arithmetic task were imposed as mental stress load.

The memory task was a computer-based task formulated by the authors. The subjects were asked to remember the combinations of randomly showed human face figures and their names each consisting of 4 Japanese characters or 9 English characters. Face figures were selected from the face database provided by William Hayward professor at The University of Hong Kong. There were 3, 4, 6, or 12 combinations of the face and the name simultaneously presented on a PC monitor for 15 sec each. After each presentation, subjects were asked to input the answers (numbers allocated in front of the names) according to their memory about the presented information from a numerical keypad within 60 sec. Therefore, the memory task consisted of 4 levels in difficulty, while level 1 presenting 3 combinations was the easiest and the level 4 presenting 12 combinations was the most difficult memory task. The order of the combinations sets was from level 1 to level 4 for each trial. Figure 1 shows a sample screen of the level 1 presentation. The task performance was calculated afterword by the percentage of the right answers in each level.

In the 10-min arithmetic task, subjects were asked to add/subtract a one-digit number to/from a two-digit number

Figure 1: Example of a level 1 presentation for the memory task

which were randomly and continuously displayed on the PC monitor and type the answer in the answer column with a numerical keypad as quickly and accurately as possible.

The accuracy rates of all of the answers were calculated for further analysis about the task performance.

2.4 Procedure

Prior to the start of the experiment, all participants were given the opportunity to familiarize themselves with all of the stress load tasks. Experiments took place in a quiet room. The room temperature was 24.5 ± 1.3 degrees C, and the humidity was 36.2 ± 6.3%. On the experiment day, as shown in Fig. 2, the participant entered the room, was seated and rested for 15 minutes. During the resting time, electrodes were attached. After the rest, a 90-second EEG measurement session took place, followed by the first VAS for the ratings on how they feel about the smell during the EEG session, even though they were under normal status of not having smelled either test odor in the first EEG measurement session. Next, the mental stress load tasks including the memory task and arithmetic task were performed. Then the second VAS and POMS were filled out. These sessions were repeated 3 times for the 3 odor samples served in random order, and the subjects smelled either of the samples keeping their eyes closed during the last 1 minute of the EEG measurement. After the 4th session of POMS was completed, the electrodes were taken off, and the participant left the experiment room.

2.5 Subjective assessment

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 first VAS was used for subjective ratings on their feelings about the odor right after smelling each sample. It comprises 5 scales including the level of how they feel "familiar", "strong", "tasty", "hate", and "not stinky" about the odor. The ratings about the two tea samples were used for the analysis.

Another VAS was used for the ratings on the mood after mental stress load sessions. This VAS comprises 8 scales on the feelings with regard to pressure, drowsiness, stress, relaxation, fatigue, security, tension and difficulty of the memory task, and all ratings in the 3 odor samples' conditions were used for further analysis.

Following the second VAS, a short version of POMS was held to assess distinct affective mood states. POMS is a popular tool used widely among psychologists and scientists from many other fields. Six identifiable mood or affective states: Tension-Anxiety (T-A), Depression-Dejection (D), Anger-Hostility (A-H), Vigor-Activity (V), Fatigue-Inertia (F), and Confusion-Bewilderment (C) can be measured and were used for analysis in this study.

2.6 Measurement

Active electrodes were attached for EEG recording at 5 locations: F3, F4, Pz, O1 and O2 according to the international 10/20 system. The two locations at frontal region (F3 and F4) were selected to detect the left and right frontal cortical activities relating to affective responses, while O1 and O2 were used for monitoring the left and right posterior cortical activities. Pz was chosen for the possibility of further study on analyzing the event-related potential (ERP) during memory task. Electrooculogram (EOG) was recorded at the left eye supra- and infra-orbitally for monitoring the ocular movements. EEG and EOG data were amplified and A/D converted by a versatile amplification unit (polymate AP1132, TEAC Corporation), and FFT was transferred offline using VitalTracer and ATAMAP II (KISSEI COMTEC CO. LTD). Filters were set for EEG at High Pass of 0.016 Hz and Low Pass of 60 Hz, for EOG at High

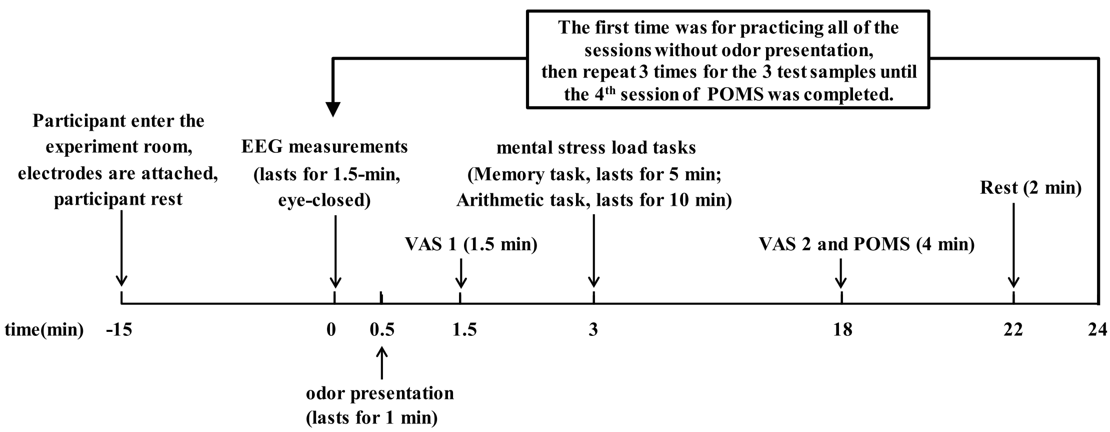

Figure 2: Procedure of the experiment

Pass of 0.16 Hz and Low Pass of 15 Hz. The sampling rate was 1000 Hz. Absolute EEG band power was calculated in the alpha1 (8-10 Hz), alpha2 (10-13 Hz), beta1 (13-20 Hz), and beta2 (20-30 Hz) bands. Data with artifacts such as ocular or body movements were excluded from further processing. For the EEG analysis, the 30-sec EEG measurement before smelling was used as baseline, and the first 30-sec EEG measurement during smelling with the participant's eyes closed were separated to 3 epochs, i.e. the first 10-sec, second 10-sec and the third 10-sec. The mean band power of the alpha 1, alpha 2, beta 1 and beta2 of the baseline and of the 3 epochs were calculated and used for the analysis..

2.7 Statistic analysis

Data were analyzed using IBM SPSS Statistics version 19. Nonparametric Friedman tests were performed to detect differences of VAS and POMS scores and task performance among the 3 odor samples. Wilcoxon Signed Ranks tests with Bonferroni correction were then carried out for the comparisons between sample treatments.

Kruskal Wallis tests were also performed to detect differences of the EEG data among the 3 odor samples compared with the baseline, which was measured at the beginning of each EEG measurement for 0.5 minutes before odor sample's presentations. Mann-Whitney's U-test with Bonferroni correction was performed for the comparisons between sample treatments.

3. RESULTS AND DISCUSSION

3.1 VAS

Figure 3 shows the result of the first VAS on feelings about the odor of the tea samples. The odor of Kouju was significantly more familiar than that of Koushun ($Z=-2.118$, $p=0.034$), and showed that it smelled slightly tastier ($Z=-1.736$, $p=0.083$) and had less feeling of hate compared with Koushun ($Z=-1.829$, $p=0.067$).

Figure 4 shows the score of the second VAS which were completed after mental tasks. The feeling of difficulty toward performing the memory task showed the trend of treatment effects ($\chi^2=4.709$, $P=0.095$) and it was slightly lower after smelling Kouju than smelling warm water ($Z=-2.307$, $p=0.021$).

From these results, the odor of Kouju showed the best score at the subjective assessments, which indicated that Kouju might be a familiar and better favorite odor than Koushun, even though it had been the first time for all the subjects to smell either of the test tea samples in this study.

3.2 POMS

All measurements in POMS were analyzed by Friedman test but no significant difference between sample treatments could be found (data not shown). That is to say, the samples used in this study showed no different effects on subjective rating about their mood after mental tasks.

3.3 Task performance

There was no significant difference could be found on the arithimatic task performances among the 3 test samples in this study.

Meanwhile, as shown in Fig. 5, difference on the accuracy rates of each level of the memory tasks was found among the 3 test samples ($\chi^2=5.871$, $P=0.053$). At level 1, which was the easiest and was performed right after the smelling session, the subjects performed a better accuracy rate in the memory task after smelling Kouju than the condition of after smelling Koushun ($Z=-2.449$, $p=0.014$). This might have been caused by the different affective effects between the two kinds of pan-fired green tea shown in the results of the first VAS. That is to say, the less favorite feelings about the odor of Koushun than Kouju, such as the lower

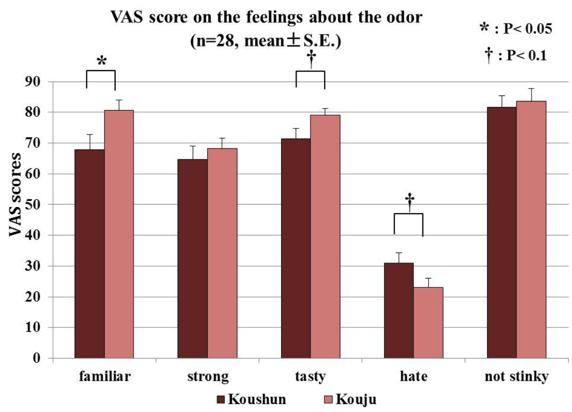

Figure 3: Results of VAS on the feelings about the tea odor samples

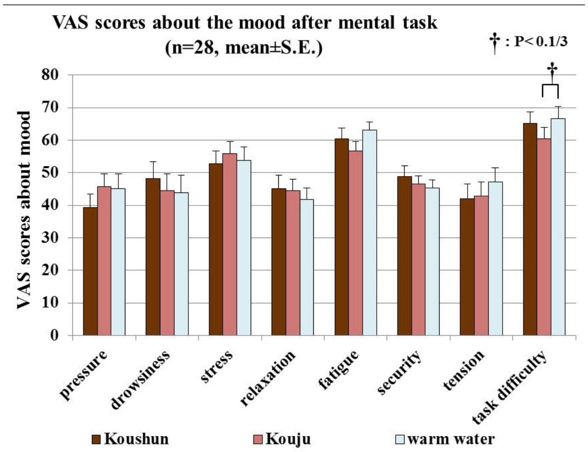

Figure 4: Results of VAS on the feelings about mood under each tea odor sample condition

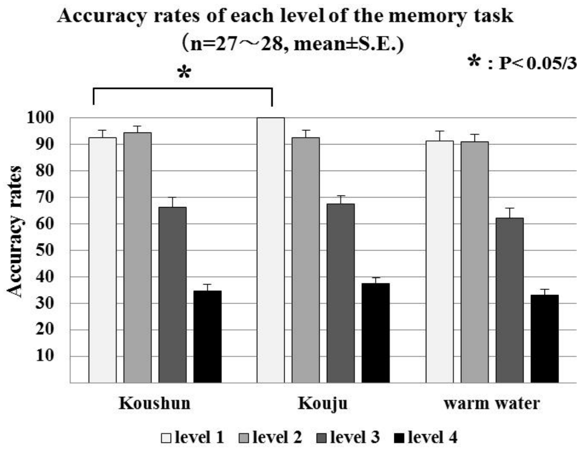

Figure 5: Results of the accuracy rates of each level of the memory tasks

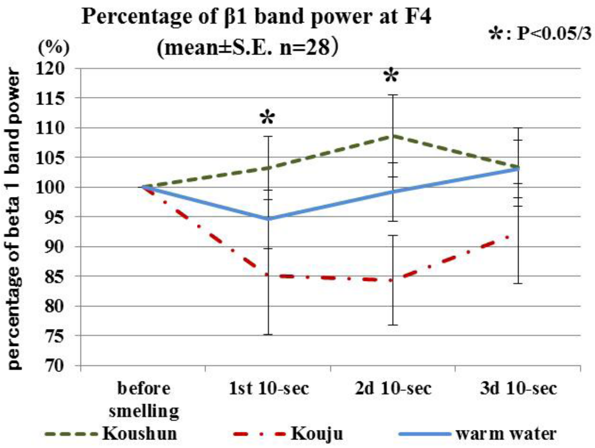

Figure 6: Results of EEG percentage of beta 1 band power at right frontal region

score of feeling "familiar" or the higher score of feeling "hate", might have lowered the easiest memory task performance right after smelling the odor. We then performed the Spearman's rank correlation coefficient tests to assess the relationship between the task performance and VAS scores. No significant correlation could be found but the level 1 accuracy rate tended to have a slight negative correlation with the score of feeling "hate" (r_s=−0.260, n=56, P=0.053).

3.4 EEG

Figure 6 shows the percentage of the EEG beta 1 power during smelling the odor samples to the baseline value calculated from the EEG power during the prior 30-second measurements before the smelling session. On the first 10-second, percentage of the beta1 power at right frontal region showed significant sample effects (χ^2=9.500, P=0.009). During the first and the second 10-second measurements, percentage of the beta1 power were significantly lower when smelling the odor of Kouju than the condition of smelling Koushun (Z=−2.869, −2.391, p=0.004, 0.017). That means, Kouju showed a different effect on EEG beta 1 activity during the first 20 second of smelling its odor.

No significant difference among test samples on the mean band power of the alpha 1, alpha 2 or beta2 on this region. The EEG power on the other regions showed no significant sample effect either in this study.

The beta band is suggested to play an important role in attention or higher cognitive functions [13] and to be related to good performance [14]. 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 [15]. However, in our study, the memory task performance of level 1 was higher with decreased band power of beta 1 at right frontal region by smelling Kouju right before completing the level 1 memory task. This might have corresponded with the different affective effect between the two kinds of tea odor showed in the results of the first VAS.

According to the frontal activation asymmetry theory, the left frontal region is associated with approach-related emotions such as happiness, joy and interest, whereas the right frontal region is associated with avoidance-related emotions, including fear, sadness and disgust [16-19]. At the same time, it is considered that the more intense the emotional experience, the greater the level of absolute frontal cortical activity [20-23]. From the VAS result in our study (Fig. 3), the odor of Kouju was more familiar and had less feeling of hate compared with Koushun. This may have caused a less anxious emotional state with a decline of right frontal cortical activity when smelling the odor of Kouju, and this positive emotional state might have then led to the better task performance, i.e. better accuracy rate at the memory task level 1.

On the other hand, both tea odor samples had low feelings of hate, as Figure 3 indicated their scores of "hate" were roughly 20-30%, the above anxiety might have due to the emotional state when the subjects were asked to wait for the odor stimulus with their eyes closed from the beginning of the EEG measurements, without knowing what kind of smell the coming odor stimulus would be like. Thus, when a more preferable odor, i.e. Kouju compared with Koushun, was presented, this anxious feeling might then somewhat relieved relatively.

The above different effects between the two kinds of pan-fired green tea may come from their different amount or the sensitivity threshold of odor components. On the other hand, it is difficult to define which component had contributed to this difference from our study. Considering that most of the aromatic components in Koushun had slightly larger amount than in Kouju (Table 1), it is more likely that not one or some of the specific components, but the combination of the total components had play an important role of the beneficial effect.

4. CONCLUSIONS

We investigated the difference of smelling two kinds of pan-fired green tea on their physical and psychological effects using EEG, subjective assessment and task performance in this study.

The results are summarized as follows:

1. The odor of Kouju was significantly more familiar than that of Koushun, and it smelled slightly tastier and had less feeling of hate from results of the subjective assessments.
2. After smelling Kouju, the accuracy rate of the memory task was significantly higher than after smelling Koushun at the easiest level of the memory task.
3. The band power of the beta 1 at right frontal region decreased with smelling Kouju.

These results indicated that the odor of Kouju may induce a positive emotion including the familiar feeling even though it was smelled for the first time; it also tended to smell tastier and slightly induce a less hate feeling. At the same time, it may also affect EEG activity and improve task performance compared with the odor of Koushun.

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