Chem. Senses 27: 225–230, 2002

Concentration Effects of Green Odor on Event-related Potential (P300) and Pleasantness

Kota Sano, Yasutami Tsuda[1], Hisanobu Sugano[1], Shuji Aou[2] and Akikazu Hatanaka[3]

Soda Aromatic Co. Ltd Fragrance Laboratory, Tokyo 103-0023, [1]MOA Health Science Foundation, Fukuoka 811-2302, [2]Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582 and [3]Department of Biochemistry, Yamaguchi University, Yamaguchi, 753-8511, Japan

Correspondence to be sent to: Kota Sano, Soda Aromatic Co. Ltd Fragrance Laboratory, 4-15-9 Nihonbashi-honcho, Chuo-ku, 103-0023 Tokyo, Japan. e-mail: kouta_sano@soda.toray.co.jp

Abstract

The effects of eight compounds, constituting the so-called 'natural green odor', including leaf alcohol, on the event-related potential (P300) were investigated. In experiments with a series of single compounds, each of these eight compounds could be characterized by an overall change consisting predominantly of an increase, a decrease or no change in the amplitude of P300, whereas in experiments with a series of two-component mixtures, noticeable synergism could not be demonstrated, contrary to our expectation. Experiments with leaf alcohol (3*Z*-hexenol) performed at two concentrations showed a significantly different degree of pleasantness and an increase or decrease in the amplitude of P300 depending on their concentration, suggesting that concentration is important in odorant-presentation studies.

Introduction

The so-called 'green odor' emanating from green leaves has been elucidated to be composed of eight odorants consisting of 6-carbon aliphatic alcohols and aldehydes including 3*Z*-hexenol (leaf alcohol) and 2*E*-hexenal (leaf aldehyde) (Hatanaka, 1999). These compounds are of plant origin that are formed by a biosynthetic reaction from the chloroplast membrane through linolenic acid (Hatanaka, 1996) and are known to have various physiological actions as plant-to-plant messengers in allelopathy, insect-attracting pheromone-like substances, bactericides and phytontids (Hatanaka, 1999). Recently, research activity has expanded to cover the field of aromachology including stress reduction (Aou *et al*., 2002a,b). We have revealed that the odor characteristics of each compound can be attributed to various factors such as differences in geometrical and positional isomerism of unsaturated bond, terminal functional groups and carbon chain lengths through our studies on the chemical structure–odor relationship of green odor (Hatanaka *et al*., 1992; Sano *et al*., 1999, 2000, 2001). The effects of the odor on the EEG were studied using the event-related potential, ERP (CNV, P300), since some compounds, mainly natural essential oils, have been shown to affect mental processes (Fukuda *et al*., 1985; Koga, 1996). The present study was therefore performed to clarify more precisely the possible effects of green odor in humans and the possible relationship between the mental effects caused by each of the green odorants and their odor characteristics, using the ERP (P300) as an indicator in our aromachological investigations.

Through their contact with plants, people come to associate green odor with nature and thereby experience pleasantness on perceiving this odor. Another purpose of the present study was to add further detailed data to our previously published data (Sugano *et al*., 1996) describing the changes in the ERP (P300) induced by green odor. As a result, we have succeeded in identifying a pattern of changes in the ERP (P300) caused by each green odorant. Furthermore, taking account the fact that natural green odor is a multi-component mixture, a series of two-component mixtures was studied with the expectation of their potential synergistic effects. In addition, the ERP (P300) was recorded at different concentrations of leaf alcohol to examine the relationship between the mental action of green odor and its concentration level and pleasantness.

Experiment 1

Materials and methods

The subjects who participated in experiment 1 were 128 healthy women aged 18–22 years, without any abnormal olfactory or auditory perception. Among these 128 women, 39 and 89 were allocated to either the experiment with a series of single compounds or that with a series of two-component mixtures, respectively. The subjects were requested to avoid using fragrant cosmetics and taking strongly smelling meals on the day of the experiment.

Green odor is known to be composed of eight compounds including *n*-hexanol, *n*-hexanal, 3*Z*-hexenol (leaf alcohol), 3*Z*-hexenal, 2*E*-hexenol, 2*E*-hexenal (leaf aldehyde), 3*E*-hexenol and 3*E*-hexenal. Thus, these eight compounds and an odorless solvent, triethyl citrate, were used as test samples. An appropriate concentration corresponding to the greatest degree of pleasantness in each of the subjects was examined preliminarily using a 10-step dilution series for 3*Z*-hexenol (leaf alcohol) and 2*E*-hexenal (leaf aldehyde), and the results obtained demonstrated that such concentrations showed a normal distribution with a peak at 0.01 and 0.1% for the former and latter, respectively. Based on the results, a concentration of 0.03% was selected as the mean of these values calculated by logarithmic transformation.

Each of the eight compounds was dissolved in triethyl citrate at a concentration of 0.03% to prepare test solutions and triethyl citrate served as the control solution (Table 2). In the experiment with a series of two-component mixtures, equal amounts of each of the test solutions were mixed (w/w%) to prepare mixed solutions of 28 combinations as shown in Table 2.

The experiment was carried out in a quiet and air conditioned room kept at ~23°C, that was, particularly, kept dim during the experiment. Subjects were asked to sit on a chair in a relaxed position keeping their eyes closed and an EEG was recorded from Fz, Cz and Pz. A sheet of filter paper (50 150 mm) was impregnated with an aliquot of test solution containing 100 mg of each odorant or control solution and held 2 cm from the tip of the nose for presentation. The EEG was recorded with a Synafit 2500 EF 2514 (NEC Corp.), with 0.5 Hz lowpass and 60 Hz highpass filters. The conventional Oddball method was applied to record ERP (P300) and acoustic stimuli were given in the form of the sound with a pressure level (SPL) of 70 dB at either 2000 or 1000 Hz, generated randomly in a ratio of 20 and 80% for the acoustic stimuli given less frequently and those given more frequently, respectively. Each of the acoustic stimuli was given at intervals of 1–1.5 s and the subjects were instructed to push a button when they heard the target sound of 2000 Hz, which occurred less frequently. P300 was obtained by averaging 20 stimuli after excluding artefacts. The experiment was designed to avoid involvement of sequence effects throughout the entire series of studies by scheduling implementation of each session so as to make intervals long enough to avoid the occurrence of olfactory fatigue.

Results

Comparison of the changes in amplitude of P300 in Fz, Cz and Pz was made between each of the eight compounds and control. Tables 1 and 2 show the overall changes in the amplitude of P300 observed with the series of single compounds and with the series of two-component mixtures, respectively. The data compiled in each table were subjected to χ^2-testing of independence after having been rearranged in a contingency table. The χ^2-value thus obtained is represented in the legend for each table (just as reference data because of small value of the expected frequency).

Table 1 Changes in P300 amplitude recorded under presentation of each of eight single compounds in comparison with control (triethyl citrate)

Test compounds[a] (0.03%)	n[b]	Change in P300 amplitude[c]		
		Increase	No change	Decrease
n-Hexanol	3	0	3	0
n-Hexanal	3	0	2	1
3*Z*-Hexenol	6	0	3	3
3*Z*-Hexenal	4	0	1	3
2*E*-Hexenol	6	2	1	3
2*E*-Hexenal	7	4	3	0
3*E*-Hexenol	6	2	1	3
3*E*-Hexenal	4	0	3	1

[a]All test solutions were prepared as 0.03% solution in odorless solvent (triethyl citrate).

[b]Number of subjects.

[c]χ^2-test results of P300 amplitude changes (reference data): Pearson χ^2-value = 21.903, d.f. =14, asymptotic significance (two-tailed) = 0.081, n = 39, Fisher's exact test: P = 0.069.

Regarding the relation between each of the test compounds and the overall predominating changes in the amplitude of P300, statistically significant association could not be identified when the data compiled in each table were analysed as a whole, whereas some of the individual samples of data compiled in Tables 1 and 2 were found to indicate a particular predominating change. More precisely, neither of the two saturated compounds elicited change in amplitude and among the unsaturated compounds, 3*Z*-hexenol (leaf alcohol) and 3*Z*-hexenal predominantly elicited a decrease, whereas 2*E*-hexenal (leaf aldehyde) predominantly elicited an increase. On the other hand, changes in the amplitude elicited by other compounds were equivocal in terms of predominance of an increase or decrease. In other words, the eight compounds did not elicit a common change, but elicited a particular change in each compound, even though the eight compounds used in the study had similar chemical structures. Figure 1 shows a representative example of decreased amplitude of P300 elicited with 3*Z*-hexenol (leaf alcohol).

Taking account of the particular importance of 3*Z*-hexenol (leaf alcohol) and 2*E*-hexenal (leaf aldehyde) among green odorants showing a unilateral change with predominantly either an increase or decrease in the study with the series of single compounds, a study was performed with

Table 2 Changes in P300 amplitude recorded under presentation of each of 28 two-component mixtures in comparison with control (triethyl citrate)

Test compounds[a] (0.03%)	n[b]	Change in P300 amplitude[c]			Test compounds[a] (0.03%)	n[b]	Change in P300 amplitude[c]		
		Increase	No change	Decrease			Increase	No change	Decrease
n-Hexanol + *n*-hexanal	3	0	1	2	3*Z*-hexenol + 2*E*-hexenol	5	1	2	2
n-Hexanol + 3*Z*-hexenol	3	1	1	1	3*Z*-hexenol + 2*E*-hexenal	6	4	2	0
n-Hexanol + 3*Z*-hexenal	3	1	0	2	3*Z*-hexenol + 3*E*-hexenol	6	2	4	0
n-Hexanol + 2*E*-hexenol	2	0	2	0	3*Z*-hexenol + 3*E*-hexenal	3	2	0	1
n-Hexanol + 2*E*-hexenal	2	0	2	0	3*Z*-hexenal + 2*E*-hexenol	2	1	0	1
n-Hexanol + 3*E*-hexenol	2	0	1	1	3*Z*-hexenal + 2*E*-hexenal	2	0	1	1
n-Hexanol + 3*E*-hexenal	4	2	0	2	3*Z*-hexenal + 3*E*-hexenol	2	1	0	1
n-Hexanal + 3*Z*-hexenol	3	1	0	2	3*Z*-hexenal + 3*E*-hexenal	2	0	0	2
n-Hexanal + 3*Z*-hexenal	2	1	1	0	2*E*-hexenol + 2*E*-hexenal	6	4	2	0
n-Hexanal + 2*E*-hexenol	2	0	2	0	2*E*-hexenol + 3*E*-hexenot	7	4	2	1
n-Hexanal + 2*E*-hexenal	2	0	1	1	2*E*-hexenol + 3*E*-hexenal	2	1	1	0
n-Hexanal + 3*E*-hexenol	2	0	0	2	2*E*-hexenal + 3*E*-hexenol	7	2	3	2
n-Hexanal + 3*E*-hexenal	2	0	0	2	2*E*-hexenal + 3*E*-hexenal	2	0	1	1
3*Z*-Hexenol + 3*Z*-hexenal	3	2	1	0	3*E*-hexenol + 3*E*-hexenal	2	0	2	0

[a]All test solutions were prepared as 0.03% solution in odorless solvent (triethyl citrate).

[b]Number of subjects.

[c]χ^2-test results of P300 amplitude changes (reference data): Pearson χ^2-value = 62.875, d.f. =54, asymptotic significance (two-tailed) = 0.191, *n* = 89.

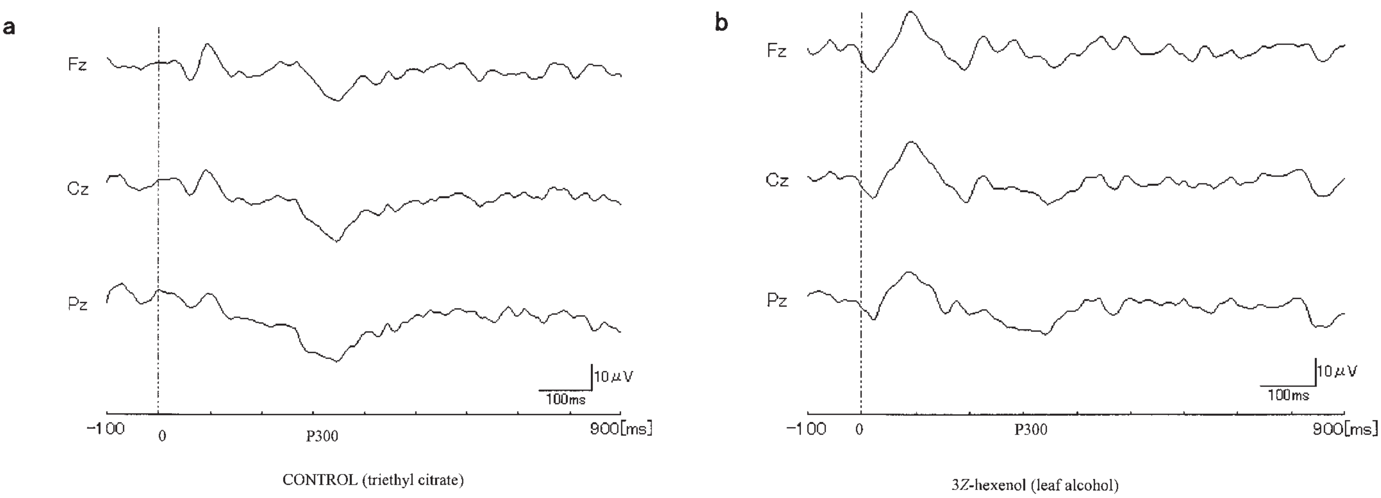

Figure 1 ERP waveforms of event-related potential (P300): an example of decrease. **(a)** Control: ERP waveforms recorded from points Fz, Cz and Pz (the sum of those collected through 20 stimuli) under presentation of odorless solvent (triethyl citrate). Stimulation was started at zero point when the first acoustic stimulus of lower frequency (2000 Hz) was given. **(b)** ERP waveforms recorded from points Fz, Cz and Pz (the sum of those collected through 20 stimuli) under presentation of 3*Z*-hexenol (leaf alcohol). Stimulation was started at zero point when the first acoustic stimulus of lower frequency (2000 Hz) was given. Decreased P300 amplitude as compared with control.

a series of two-component mixtures of each of 3*Z*-hexenol and 2*E*-hexenal with other odorants. This showed that two of the two-component mixtures—2*E*-hexenal with 3*Z*-hexenol and 2*E*-hexenal with 2*E*-hexenol—predominantly elicited an increase. Overall evaluation of the data obtained from the 89 subjects, however, did not demonstrate any synergistic effects of mixtures, contrary to our expectation prior to the study.

Experiment 2

Materials and methods

The procedure and method were the same as in experiment 1. The subjects who participated in experiment 2 were 34 healthy women aged 18–22 years.

Among the eight compounds composing green odor, 3*Z*-hexenol (leaf alcohol) was selected for further detailed investigation, as the dominant odorant of green odor. Concentrations of 10 and 0.1% (w/w%) were selected based on the outcomes of preliminary examination of pleasantness. The test compound was dissolved in triethyl citrate at concentrations of 10 and 0.1% to prepare test solutions; triethyl citrate served as the control solution.

Recording of P300 was carried out in the same manner as in experiment 1 in terms of the environment, equipment and procedure; measurements of the amplitude of P300 derived from Pz were averaged over the entire 20 stimuli and, after adjustment of the baseline, data contaminated with artefacts were excluded. Thus, cleaned-up data were subjected to analysis by paired *t*-test. Statistical analysis was performed using SPSS software. After completion of EEG recording, odor intensity (0–5), degree of pleasantness (–4 to +4) and impression of odor were surveyed through an interview. Odor intensity and the degree of pleasantness were each assessed based on the '6-rating classification of odor intensity' and '9-rating classification of pleasantness/unpleasantness', respectively, which are commonly used by the Ministry of the Environment, as shown in Table 3.

Table 3 '6-Rating classification of odor intensity'/'9-Rating classification of pleasantness/unpleasantness'

6-Rating classification of the odor intensity	9-Rating classification of the pleasantness/unpleasantness
0: Odorless	–4: Extremely unpleasant
1: Barely perceptible odor	–3: Highly unpleasant
2: Faint but source-discriminative odor	–2: Unpleasant
	–1: Slightly unpleasant
3: Easily perceptible odor	0: Equivocally pleasant
4: Strong odor	+1: Slightly pleasant
5: Extremely strong odor	+2: Pleasant
	+3: Highly pleasant
	+4: Extremely pleasant

Assessment scales used in survey questions on the odor intensity and the extent of pleasantness.

Results

Among the data obtained from the 34 subjects, analysis was performed on the data obtained from 31 subjects remaining after excluding three subjects in whom P300 could not be identified. For analysis, the amplitude of P300 derived from Pz was compared for each of 10% 3*Z*-hexenol, 0.1% 3*Z*-hexenol and triethyl citrate (control). As shown in Figure 2, for 10% 3*Z*-hexenol and 0.1% 3*Z*-hexenol, the amplitude of P300 was significantly smaller ($P < 0.01$) as compared with control. The outcomes agreed with those observed in experiment 1 in that 3*Z*-hexenol predominantly elicited a decrease in amplitude. Furthermore, it was found that the decrease in P300 amplitude was significantly larger ($P < 0.05$) with 0.1% 3*Z*-hexenol as compared with 10% 3*Z*-hexenol. Figure 3 shows the results of survey questions asked after completion of EEG recording, showing a significantly greater degree of pleasantness ($P < 0.01$) experienced with 0.1% 3*Z*-hexenol than with 10% 3*Z*-hexenol. The results demonstrated that the degree of pleasantness experienced with the same compound varied markedly depending on the concentration. The 31 subjects were stratified into three subgroups: a group of 26 subjects who rated 0.1% 3*Z*-hexenol more pleasant smelling than 10% 3*Z*-hexenol; a group of two subjects who rated 0.1% 3*Z*-hexenol and 10% 3*Z*-hexenol equally pleasant smelling; and a group of three subjects who rated 10% 3*Z*-hexenol more pleasant smelling than 0.1% 3*Z*-hexenol. The values of P300 potential with 10% 3*Z*-hexenol and 0.1% 3*Z*-hexenol were compared among these three subgroups. In the subgroup of 26 subjects who rated 0.1% 3*Z*-hexenol more pleasant smelling than 10% 3*Z*-hexenol, a finding of a lower potential with 0.1 than 10% was obtained in 20 subjects (~77%), a comparable potential with both 0.1 and 10% in one (~4%) and a higher potential with 0.1 than 10% in five (~19%), whereas in the subgroup of three subjects who rated 10% 3*Z*-hexenol as more pleasant smelling than 0.1% 3*Z*-hexenol, a finding of a lower potential with 10 than with 0.1% was obtained in all three subjects.

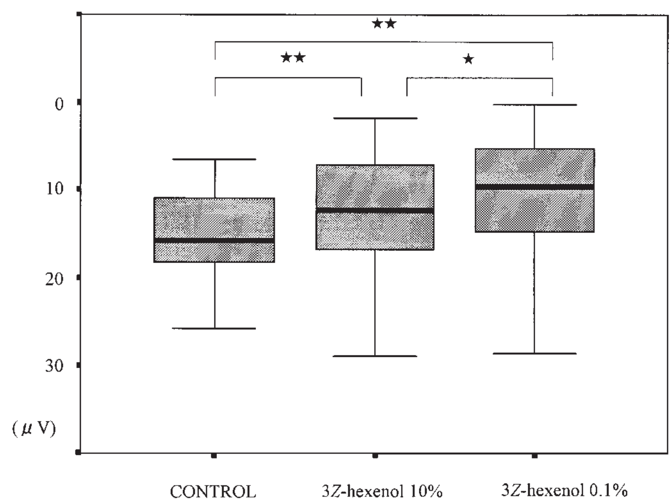

Figure 2 Concentration-dependent changes in P300. Median, 50%, maximum and minimum values are depicted. Vertical axis represents the value of P300 potential derived from Pz. *$P < 0.05$; **$P < 0.01$.

General discussion

The results of experiment 1 revealed that the eight compounds composing green odor can be classified into three categories: those that increase, those that do not change and those that decrease the amplitude of P300, regardless of their high similarity in chemical structure and odor characteristics. Experience of various feelings of, for example, refreshment and relaxation in association with perception of a natural green odor reflects the fact that odors of natural origin are due to multi-component mixture of compounds,

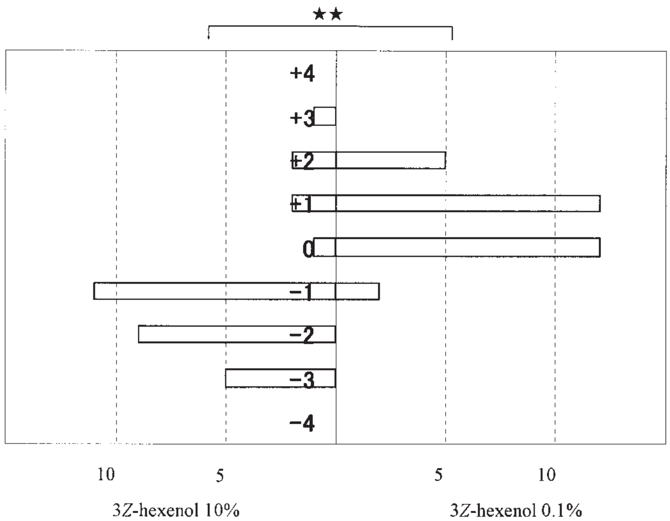

Figure 3 Concentration-dependent changes in pleasantness. Vertical axis represents the rating scale for the extent of pleasantness/unpleasantness. The figure depicts the number of subjects corresponding to each rating scale. Statistically significant difference in the extent of pleasantness between 10% and 0.1% solutions. $^{**}P < 0.01$.

each with a different mental action. Our findings that saturated compounds did not elicit any change in amplitude, while many unsaturated compounds elicited changes that were predominantly either an increase or decrease help us to understand that with respect to differences in chemical structure, unsaturated-bond-containing compounds favor perception of an odor in humans.

The results of experiment 2 revealed that the amplitude of P300 was significantly decreased with 3*Z*-hexenol (leaf alcohol) Although various interpretations have been proposed for the changes in the amplitude of P300, a widely accepted interpretation is that its change reflects possible influence on the cognitive function (Intriligator and Polich, 1994). A decrease in the amplitude of P300 is considered to reflect the onset of a kind of sedative action (Yagyu *et al.*, 1992; Yamadera *et al.*, 1996) and, thus, the series of compounds including 3*Z*-hexenol is considered to be involved in the feeling of relaxation induced by green odor. An interesting finding obtained through the results of experiment 2 was that the amplitude of P300 was certainly decreased with 3*Z*-hexenol; nevertheless, the decrease was not necessarily larger in response to a higher concentration. The finding that the amplitude-decreasing effect was larger with a concentration of 0.1 than with 10% agreed with the findings obtained in survey questions on the degree of pleasantness, as shown by the findings that the decrease in amplitude of P300 was greater with 3*Z*-hexenol at the concentration corresponding to a greater degree of pleasantness. A report describing that, when different substances were compared, not pleasant but unpleasant odors elicited decrease in the amplitude of P300 is available (Koga, 1996), whereas, in our present study in which two different concentration levels of an identical substance were compared, the concentration level corresponding to higher degree of pleasantness was found to elicit larger decrease in the amplitude of P300. On the other hand, the findings showed that the amplitude of P300 was generally lower with 0.1% 3*Z*-hexenol as compared with 10% 3*Z*-hexenol (as shown in Figure 2), whereas, in the subgroup of three subjects who rated the 3*Z*-hexenol solution more pleasant smelling at a concentration of 10% than at 0.1%, the potentials were lower with 10% 3*Z*-hexenol as compared with 0.1% 3*Z*-hexenol in all three of these subjects. These findings suggest that the amplitude-decreasing effect does not depend on the concentration but rather on the degree of pleasantness, in that a pleasant smell may enhance and an unpleasant smell may attenuate the sedative effect. Consequently, green odor has been demonstrated to constitute an element of aromachology by bringing about stress reduction in humans. Further study to investigate any relationship between EEG, particularly alpha wave and ERP (P300) each recorded under comparative conditions, is to be planned.

In recent years, selection of appropriate concentration levels of test samples in designing study protocols has been suggested to be a matter of importance, through investigations on the relation between odor and EEG conducted at various research institutions. The present paper suggests that data on the degree of pleasantness is important as a rationale for concentration selection.

Acknowledgements

We gratefully acknowledge Dr Nobushige Sato, Emeritus Professor and Dr Shigeki Kitajima, Associate Professor at the University of Occupational and Environmental Health, Japan, for their cooperation and recruitment of volunteers for the study.

References

Aou, S., Matsunaga, Y., Nakagawa, T., Kubo, K, Hori, T., Oomura, Y., Sano, K. and **Hatanaka, A.** (2002a) *Effects of green odor on rat behaviors.* Physiol. Behav.

Aou, S., Mizuno, M., Kamikawa, H., Kanemitsu, Y., Abe, M., Kubo, K., Li, X.L., Hori, T., Sano, K. and **Hatanaka, A.** (2002b) *Effects of green odor on sensorimotor function and nociception in human.* Physiol. Behav.

Fukuda, H., Torii, S., Kanemoto, H., Takino, R., Miyauti, S. and **Hamauzu, Y.** (1985) *Effect of odors on contingent negative variation (CNV).* In Kimura, S., Miyoshi, A. and Shimada, I. (eds), Proceedings of the 19th Japanese Symposium on Taste and Smell. Keikosha, Sendai, pp. 65–68.

Hatanaka, A. (1996) *The fresh green odor emitted by plants.* Food Rev. Int., 12, 303–350.

Hatanaka, A. (1999) *Biosynthesis of so-called 'green odor' emitted by green leaves.* In Barton, D. and Nakanishi, K. (eds), Comprehensive Natural Products Chemistry, 9 Vols. Elsevier Science, Vol. 1, pp. 83–116.

Hatanaka, A., Kajiwara, T. and **Horino, H.** (1992) *Odor–structure relationships in* n-*hexenols and* n-*hexenals*. Z. Naturforsch., 47c, 183–189.

Intriligator, J. and **Polich, J.** (1994) *On the relationship between background EEG and the P300 event-related potential*. Biol. Psychol., 37, 207–218.

Koga, Y. (1996) *Influence of coffee aroma on brain function: results from studies of rCBF and ERPs.* In Ogura, C., Koga, Y. and Shimokochi, M. (eds), Recent Advances in Event-related Brain Potential Research. Elsevier, Amsterdam, pp. 372–378.

Sano, K., Suzuki, J. and **Hatanaka, A.** (1999) *Chemical structure–odor relationships of 'green odor' in green leaves.* Jpn J. Taste Smell Res., 6, 461–464.

Sano, K., Suzuki, J. and **Hatanaka, A.** (2000) *Chemical structure–odor relationships of 'green odor' in fruits.* Jpn J. Taste Smell Res., 7, 473–476.

Sano, K., Suzuki, J. and **Hatanaka, A.** (2001) *Chemical structure–odor relationships of 'green odor' affected by terminal functional groups.* Jpn J. Taste Smell Res., 8, 419–422.

Sugano, H., Uchida, S., Sato, S., Hatanaka, A. and **Sano, K.** (1996) *Effects of green odor on event related potential* (P300). Jpn J. Taste Smell Res., 3, 672–674.

Yagyu, T., Saito, N., Nobuhara, K., Ohashi, Y., Fukushima, M., Okajima, Y., Su, G. and **Saito, M.** (1992) *Changes of EEG basic activity and ERPP300 component due to the stimulation of odorant in human*. Clin. Electroencephal., 34, 498–501.

Yamadera, H., Tsukahara, Y., Kato, M. and **Okuma, T.** (1996) *Zopiclone pharmacodynamics: by monitoring event-related potentials and psychometric scores*. Nihon Shinkei Seishin Yakurigaku Zasshi, 16, 145–149 (in Japanese).

Accepted November 28, 2001