Chem. Senses 26: 281–292, 2001

Effects of Chiral Fragrances on Human Autonomic Nervous System Parameters and Self-evaluation

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Abstract

The effects of chiral fragrances (enantiomers of limonene and carvone) on the human autonomic nervous system (ANS) and on self-evaluation were studied in 20 healthy volunteers. Each fragrance was administered to each subject by inhalation using an A–A–B design. Individuals were tested in four separate sessions; in one session one fragrance was administered. ANS parameters recorded were skin temperature, skin conductance, breathing rate, pulse rate, blood oxygen saturation and systolic as well as diastolic blood pressure. Subjective experience was assessed in terms of mood, calmness and alertness on visual analog scales. In addition, fragrances were rated in terms of pleasantness, intensity and stimulating property. Inhalation of (+)-limonene led to increased systolic blood pressure, subjective alertness and restlessness. Inhalation of (–)-limonene caused an increase in systolic blood pressure but had no effects on psychological parameters. Inhalation of (–)-carvone caused increases in pulse rate, diastolic blood pressure and subjective restlessness. After inhalation of (+)-carvone increased levels of systolic as well as diastolic blood pressure were observed. Correlational analyses revealed that changes in both ANS parameters and self-evaluation were in part related to subjective evaluation of the odor and suggest that both pharmacological and psychological mechanisms are involved in the observed effects. In conclusion, the present study indicates that: (i) prolonged inhalation of fragrances influences ANS parameters as well as mental and emotional conditions; (ii) effects of fragrances are in part based on subjective evaluation of odor; (iii) chirality of odor molecules seems to be a central factor with respect to the biological activity of fragrances.

Introduction

The main constituents of fragrances and essential oils (natural mixtures of fragrance compounds) are volatile monoterpenes which contribute to flavors and aromas in food and pharmaceutical preparations. Also, they are widely used in perfumery. In folk medicine as well as in phytotherapy essential oils have been and are still used as therapeutic agents, for example as a carminative, laxative or as a digestion aid and especially against infections of the respiratory tract. In aromatherapy [precisely defined elsewhere (Buchbauer, 1996; Buchbauer and Jirovetz, 1994)] some essential oils have been used successfully in the treatment of depression, anxiety and some forms of cognitive disorders as well as in insomnia and stress-induced ailments. Recently other authors (Crowell *et al.*, 1992; Haag *et al.*, 1992) have demonstrated that limonene exhibits both chemopreventive and therapeutic effects against chemically induced mammary tumors in rats. Carvone has also been associated with chemopreventive activity, since it has been found to induce the detoxifying enzyme glutathione *S*-transferase in several mouse target tissues (Zheng *et al.*, 1992). Yamada *et al.* (Yamada *et al.*, 1994) reported on the biological activity of linalool. In their studies linalool was found to have anticonvulsant activity in mice. Moreover, other groups (Buchbauer *et al.*, 1993; Elisabetsky *et al.*, 1995; Hardy *et al.*, 1995) were able to show a sedative effect of linalool. 1,8-Cineole increased the cerebral blood supply upon inhalation (Našel *et al.* 1994). Finally, the influence of chirality on pharmacological properties has also been discussed (Sugawara *et al.*, 1998; Lis-Balchin *et al.*, 1999; Jäger *et al.*, 2000).

Many researchers investigating odor effects on human behavior have detected immediate physiological changes in response to olfactory stimulation in parameters such as blood pressure (Suzuki and Aoki, 1994), muscle tension (Schwartz, 1979), pupil dilation (Steiner *et al.*, 1977), blink magnitude (Ehrlichman *et al.*, 1997), skin temperature, skin blood flow, electrodermal activity, heart rate (Brauchli *et al.*, 1995; Alaoui-Ismaïli *et al.*, 1997) and brain wave patterns (Lorig and Schwartz, 1988; Torii *et al.*, 1988; Van Toller *et al.*, 1993). Many of these reports have focused on the

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influence of qualitative properties of the stimuli, especially hedonic tone, on the results. However, the role of other psychological parameters, such as self-reports, has rarely been investigated. Moreover, to our knowledge no data about longer lasting effects of fragrances on the autonomic nervous system (ANS) and about the influence of chirality of the substances on these effects are available so far.

Therefore, the main objectives of the present study were: (i) to determine the effects of fragrance compounds on ANS parameters as well as on behavioral measures on an activating–sedating (relaxing) dimension after prolonged inhalation in healthy volunteers; (ii) to explore differences between enantiomers of fragrance compounds with respect to their effects on ANS parameters and on mental and emotional conditions; (iii) to elucidate whether the effects are solely dependent on the subjectively perceived quality of the odor or if there exists some kind of pharmacological mechanism.

Materials and methods

Subjects and fragrance compounds

Ten male and 10 female healthy volunteers aged between 18 and 36 years (mean age 24.30 ± 3.98 years) took part in the experiments. Each subject was tested in four individual sessions which took place 1 week apart but always at the same time of day. In each session each subject inhaled one of four fragrance compounds (all products of Aldrich Chemical Co., Milwaukee, WI): *R*-(+)-limonene in the first session, *S*-(–)-limonene in the second, *R*-(–)-carvone in the third and *S*-(+)-carvone in the fourth session. The sequence of administration was not randomized. Twenty-four hours prior to the beginning of each experiment subjects had to abstain from food and beverages containing the substances tested, e.g. oranges, lemons, Ceylon cinnamon, sassafras, eucalyptus, caraway, fennel and chewing gum, as well as from tea, coffee and alcoholic beverages. Enantiomers of limonene and carvone were chosen as olfactory stimuli since the pharmacokinetic properties of these antipodes have recently been studied by Buchbauer's group (Mayer, 1999; Jäger *et al.*, 2000). All subjects gave written informed consent to all aspects of the study (Viennese Ethic Commission's permissions no. 324/96, 419/98).

Fragrance delivery

Odorants were administered by inhalation via a fragrance delivery system developed by our group which consisted of an air tank, a drug nebulization device (Inhalette; Dräger, Vienna, Austria) and a breathing mask (B+P Beatmungsprodukte GmbH, Neunkirchen, Germany). The air flow from the tank was set to 12 l/min. The nebulizer was filled with 500 mg fragrance/subject. Subtraction of the weight of the nebulizer after inhalation from the weight before inhalation gave the consumption of substance.

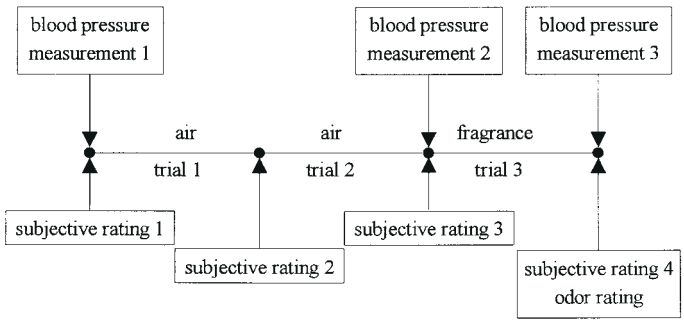

Figure 1 Experimental design.

Experimental design

The experimental design is shown in Figure 1. One session consisted of three trials of 30 min each. At the beginning of the first trial as well as at the end of each trial subjective mental and emotional conditions were assessed with visual analog scales (VAS). In addition, subjective ratings of the perceived odor were measured at the end of the third trial. ANS parameters were recorded continuously during each trial. Blood pressure was measured at the beginning of the first as well as at the end of the second and third trials. In the first trial individual basic levels of both ANS parameters and subjective ratings were assessed. The second trial served as a control for influences of the experimental set-up. During these trials the nebulizer was filled with water and subjects inhaled pure air. Fragrances were presented continuously during the third trial.

Autonomic parameter recording

Five ANS parameters were recorded simultaneously and in real time: skin temperature (ST), skin conductance (non-specific fluctuations of SC), breathing rate (BR), pulse rate (PR) and blood oxygen saturation (BOS). All parameters were measured using MP100WSW hardware (Biopac Systems, Santa Barbara, CA) and Acq*Knowledge* software (v.3.2.6; Biopac Systems). Sampling rate was 20 Hz. An example of a recording is given in Figure 2. Systolic and diastolic blood pressure (SBP and DBP) were measured three times throughout the experiment.

Skin conductance (SC)

SC was recorded using a GSR100B amplifier and Ag/AgCl finger electrodes (Biopac TSD103A) by the constant voltage (0.5 V) technique. Electrodes were filled with conductive gel and placed on the first phalanx of the middle and the index finger of the non-dominant hand with non-caustic adhesive tape.

Skin temperature (ST)

ST was measured with a SKT100B amplifier and a fast response thermistor (Biopac TSD102A). The sensor was placed on the middle of the back of the non-dominant hand with non-caustic adhesive tape.

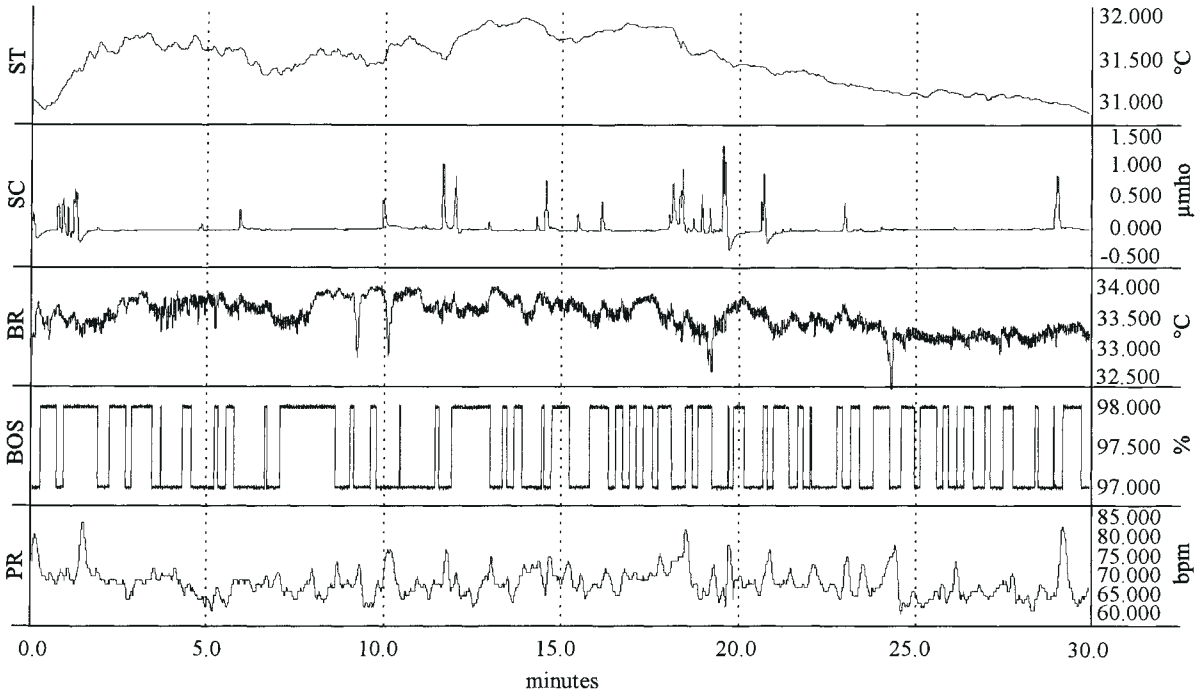

Figure 2 Example recording [third trial in a (–)-limonene session]. ST, skin temperature; SC, skin conductance; BR, breathing rate; BOS, blood oxygen saturation; PR, pulse rate.

Pulse rate (PR) and blood oxygen saturation (BOS)

PR and BOS were assessed using a Pulse Oximeter Module (OXY100A) and a photoelectrical transducer (Biopac TSD123B). The non-invasive transducer was placed on the first phalanx of the ring finger of the non-dominant hand with non-caustic adhesive tape.

Breathing rate (BR)

BR was measured using a SKT100B amplifier and a TSD102D surface temperature thermistor probe which registers breathing cycles on the basis of the difference in temperature between inhaled and exhaled air. The probe was placed at the entrance of the left nostril with non-caustic adhesive tape.

Blood pressure (SBP and DBP)

SBP and DBP were measured in the left arm by sphygmomanometry using an automated system (Hartmann Digital HG160; Paul Hartmann AG, Heidenheim, Germany).

VAS

VASs were used to assess subjective mental and emotional conditions. They consisted of 100 mm lines for three items: 'mood', 'calmness' and 'alertness'. Each subject was asked to mark his or her feeling for each item between the two possible extremes: alert (on the left) and tired (on the right) for the item 'alertness', calm (on the left) and restless (on the right) for the item 'calmness' and cheerful (on the left) and bad tempered (on the right) for the item 'mood'. After the third trial subjects additionally rated the perceived odor in terms of pleasantness (pleasant–unpleasant), intensity (weak–strong) and effect (stimulating–tiring) on 100 mm VASs.

Procedure

The experiments were conducted in a bright and quiet room. Room temperature was 21–24°C. Subjects were seated in a semi-reclined position, providing easy access to attach the electrodes. They were aware that a fragrance was to be delivered through a facial mask, but did not know when.

Upon arrival subjects were interviewed about their personal data, i.e. name, age, sex, weight and height. In addition, subjects were asked about their dominant hand by means of the Edinburgh Handedness Inventory (Oldfield, 1971) and about their mental and emotional condition on VASs. After completion of the interview and rating scales, fat free body mass (by bioelectrical impedance analysis), SBP and DBP were measured. Subsequently, subjects were informed about the proceedings. Electrodes were placed on suitable positions as stated above. Then the breathing mask was attached to the subject's face to cover the nose and mouth and recording of the ANS parameters was started. After completion of each trial subjects were allowed to remove the breathing mask and the rating scales were presented. In addition, SBP and DBP were measured after the second and third trials.

Data analysis

Data reduction

All ANS parameters were computed per individual by trial using Acq*Knowledge* software. Each trial was divided into six intervals of 5 min. Mean values of ST, PR and BOS within each interval were obtained for each subject. BR was determined by counting the number of breaths per interval and was subsequently converted into breaths per min. Similarly, the rate of non-specific fluctuations of SC was determined by counting the peaks per interval and was then converted into non-specific fluctuations per min. The minimum amplitude of a peak to be counted was 0.01 μmho and was in compliance with traditional recommendations (Stern *et al.*, 1980). For every subject and every parameter the mean value in a given interval (e.g. the first) in the second trial was subtracted from the mean value in the corresponding interval (i.e. the first) in the first trial, resulting in six individual difference scores between the first and second trials. These were averaged to give one individual inter-trial difference score between the first and second trials. The same procedure was used to determine individual inter-trial difference scores between the second and third trials. Also, difference scores between blood pressure measurements 1 and 2 and measurements 2 and 3 were calculated for each subject.

For mental and emotional condition and fragrance ratings on each scale the distance of the mark from the left- hand side was measured in millimeters. Individual difference scores between ratings 1 and 2, 2 and 3 and 3 and 4 were calculated for each item.

Statistical analysis

Systat 7.0 (SPSS Inc., Chicago, IL) was used for data analysis. Non-parametric Wilcoxon signed rank tests were used given the rather small sample size and the high inter-individual variability observed. Within sessions the effects of the fragrances on ANS parameters and ratings of mental and emotional condition were determined by comparing the difference scores. Comparisons of the difference scores between sessions were made to evaluate differences between enantiomeric pairs. Consumption of fragrance was compared between enantiomeric pairs by means of matched samples *t*-tests.

Correlations between ANS parameters, self-evaluation and fragrance ratings were studied by means of the Pearson product moment correlation and the Spearman rank order correlation.

Results

Autonomic nervous system parameters

Pulse rate (PR)

Mean inter-trial difference scores for PR for all sessions are shown in Figure 3. In the (+)-carvone session positive difference scores between the first and second trials [difference (12)] as well as between the second and third trials [difference (23)] were found, i.e. a decrease in PR in the second trial (as compared with the first) and in the third trial (as compared with the second). Comparison of the inter-trial difference scores revealed a trend towards a smaller decrease in PR during the inhalation of (+)-carvone in the third trial ($P = 0.094$).

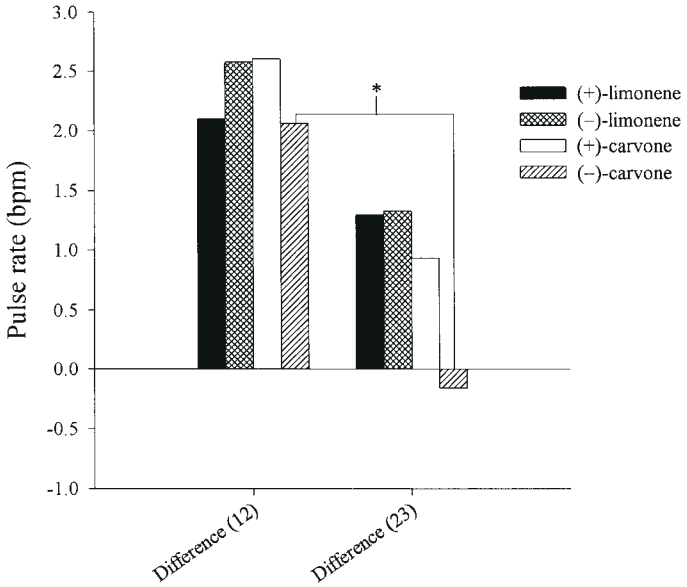

Figure 3 Mean inter-trial PR difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between trials 1 and 2; difference (23), difference score between trials 2 and 3; $*P \leq 0.05$.

In the (–)-carvone session a positive difference score between the first and second trials, i.e. a decrease in the second trial (as compared with the first), was in significant contrast to a negative difference score between trials 2 and 3, i.e. an increase in PR during inhalation of (–)-carvone in the third trial ($P = 0.033$).

Comparison of the inter-trial difference scores between the carvone sessions revealed no significant differences [difference (12), $P = 0.709$; difference (23), $P = 0.811$].

In both limonene sessions positive difference scores between the first and second trials [difference (12)] as well as between the second and third trials [difference (23)] were found, i.e. a decrease in PR in the second trial (as compared with the first) and in the third trial (as compared with the second). Comparison of the inter-trial difference scores within sessions revealed no significant effect of any enantiomer of limonene on PR [(+)-limonene, $P = 0.205$; (–)-limonene, $P = 0.192$].

Comparison of the inter-trial difference scores between the limonene sessions revealed no significant differences [difference (12), $P = 0.543$; difference (23), $P = 0.768$].

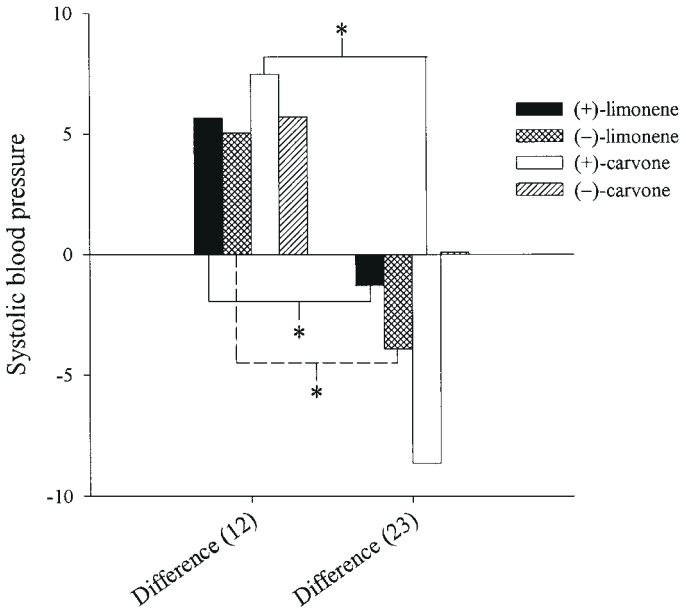

Figure 4 Mean inter-trial SBP difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between trials 1 and 2; difference (23), difference score between trials 2 and 3; $*P \leq 0.05$.

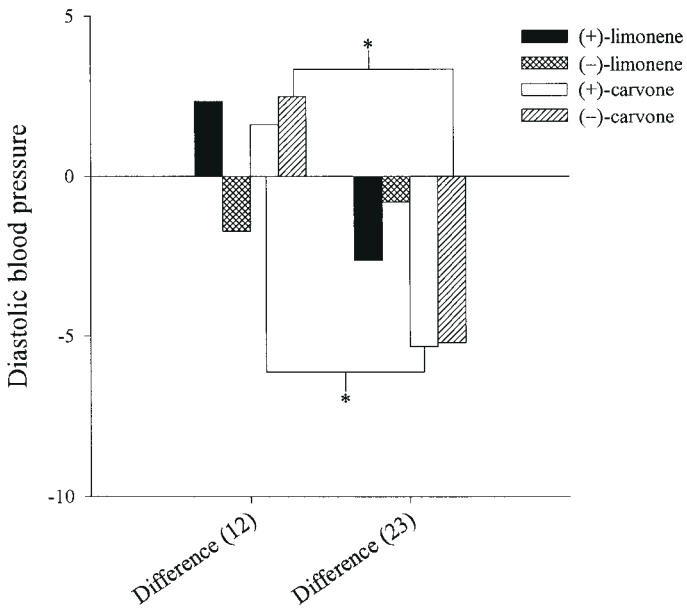

Figure 5 Mean inter-trial DBP difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between trials 1 and 2; difference (23), difference score between trials 2 and 3; $*P \leq 0.05$.

Systolic blood pressure (SBP)

Mean difference scores for SBP for all sessions are presented in Figure 4. In all sessions positive difference scores between measurements 1 and 2 [difference (12)] were found, i.e. a decrease in SBP at the end of the second trial (as compared with the beginning of the first). Difference scores between the second and third measurements [difference (23)] were negative, i.e. an increase in SBP at the end of the third trial (as compared with the second) was observed for all sessions, except for the (–)-carvone session, in which a very small positive difference score was found. In the (+)-limonene ($P = 0.036$), the (–)-limonene ($P = 0.003$) and the (+)-carvone ($P = 0.001$) sessions the difference scores were in significant contrast. However, no significant effect of (–)-carvone on SBP was found ($P = 0.104$).

Comparison of the difference scores (23) between the carvone sessions showed a trend towards an increase in SBP during the inhalation of (+)-carvone as opposed to a very small decrease during inhalation of (–)-carvone ($P = 0.061$). No significant difference was found between the difference scores (12) ($P = 0.865$).

No significant differences were found between the limonene sessions [difference (12), $P = 0.793$; difference (23), $P = 0.214$).

Diastolic blood pressure (DBP)

Figure 5 shows mean difference scores for DBP for all sessions. In both carvone sessions positive difference scores between the first and second measurements [difference (12)], i.e. a decrease in DBP at the end of the second trial (as compared with the beginning of the first), and negative difference scores between the second and third measurements [difference (23)], i.e. an increase in DBP at the end of the third trial (as compared with the second), were found. Within both sessions the difference scores were in significant contrast [(+)-carvone, $P = 0.020$; (–)-carvone, $P = 0.018$].

No significant differences were found between the carvone sessions [difference (12), $P = 0.950$; difference (23), $P = 0.602$].

In the (+)-limonene session a positive difference score between the first and second measurements [difference (12)], i.e. a decrease in DBP at the end of the second trial (as compared with the first), and a negative difference score between the second and third measurements [difference (23)], i.e. an increase in DBP at the end of the third trial (as compared with the second), were observed. In the (–)-limonene session both difference scores [difference (12) and difference (23)] were negative. However, no significant effect of any enantiomer of limonene on DBP was found [(+)-limonene, $P = 0.161$; (–)-limonene, $P = 0.904$].

Comparison of the difference scores between the limonene sessions revealed no significant differences [difference (12), $P = 0.559$; difference (23), $P = 643$].

Skin temperature (ST), skin conductance (SC), breathing rate (BR) and blood oxygen saturation (BOS)

No significant effects of the fragrances on ST, on the rate of non-specific fluctuations of SC, on BR or on BOS were found ($P > 0.1$ for all; data not shown).

Mental and emotional condition

Alertness

Mean difference scores between ratings 1 and 2 [difference (12)], 2 and 3 [difference (23)] and 3 and 4 [difference (34)] of subjective alertness for all sessions are depicted in Figure 6. In all sessions negative difference scores between the first and second ratings were found, i.e. a decrease in subjective alertness during the first trial. However, positive difference scores between ratings 2 and 3 as well as between ratings 3 and 4, i.e. an increase in subjective alertness during the second as well as third trials, were observed.

In the (–)-limonene session comparison of difference scores (12) and (23) revealed a significant decrease in alertness during the first trial as opposed to a slight increase in subjective alertness during the second trial ($P = 0.011$). Comparison of the difference scores (12) and (34) revealed a significant increase in subjective alertness during the inhalation of both (–)-limonene and (+)-limonene ($P = 0.005$ and $P = 0.010$, respectively). However, no significant difference was found between difference scores (23) and (34) in any limonene session [(+)-limonene, $P = 0.187$; (–)-limonene, $P = 0.626$].

Comparison of the difference scores (12) between the limonene sessions revealed that subjects felt significantly less alert in the (–)-limonene session than in the (+)-limonene session ($P = 0.015$). No significant differences between the limonene sessions were found for difference scores (23) and (34) [difference (23), $P = 0.931$; difference (34), $P = 0.154$].

In the (–)-carvone session comparison of difference scores (12) and (23) showed a trend towards tiredness during the first trial as opposed to a small increase in subjective alertness during the second trial ($P = 0.057$). Comparison of difference scores (12) and (34) revealed a significant increase in alertness during the inhalation of (–)-carvone ($P = 0.004$). However, no significant difference was found between difference scores (23) and (34) ($P = 0.184$). No significant effects of (+)-carvone on subjective alertness were found [difference (12) versus difference (23), $P = 0.179$; difference (12) versus difference (34), $P = 0.575$; difference (23) versus difference (34), $P = 0.955$].

Comparison of difference scores (12), (23) and (34) between the carvone sessions revealed no significant differences [difference (12), $P = 0.191$; difference (23), $P = 0.538$; difference (34), $P = 0.322$].

Calmness

Mean difference scores between ratings 1 and 2 [difference (12)], 2 and 3 [difference (23)] and 3 and 4 [difference (34)] of subjective calmness for all sessions are shown in Figure 7. In all sessions positive difference scores between ratings 1 and 2 were found, i.e. an increase in subjective calmness during the first trial. Also, positive difference scores between ratings 2 and 3 were observed in all sessions, except for the (+)-carvone session, in which a negative difference score was found, i.e. a decrease in subjective calmness during the second trial. In contrast, negative difference scores between ratings 3 and 4 (a decrease in subjective calmness during inhalation of the substances in trial 3) were found in all sessions, except for the (–)-limonene session, in which a positive difference score was observed.

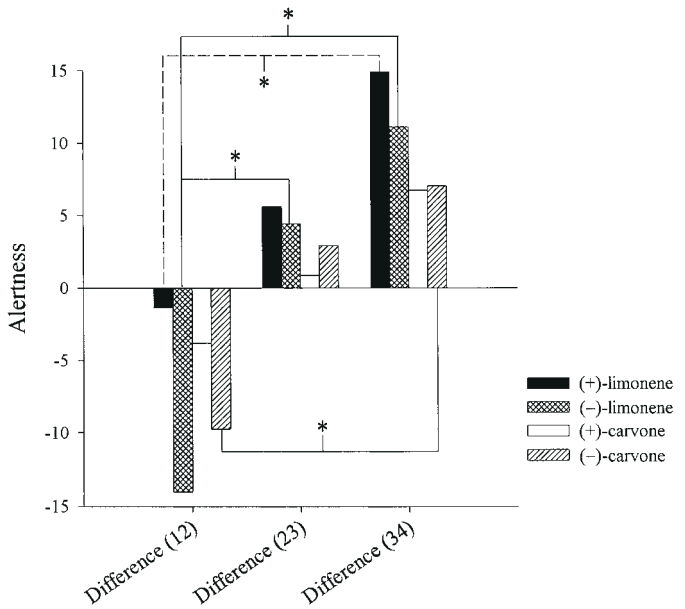

Figure 6 Mean inter-trial subjective alertness difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between ratings 1 and 2; difference (23), difference score between ratings 2 and 3; difference (34), difference score between ratings 3 and 4; $*P \leq 0.05$.

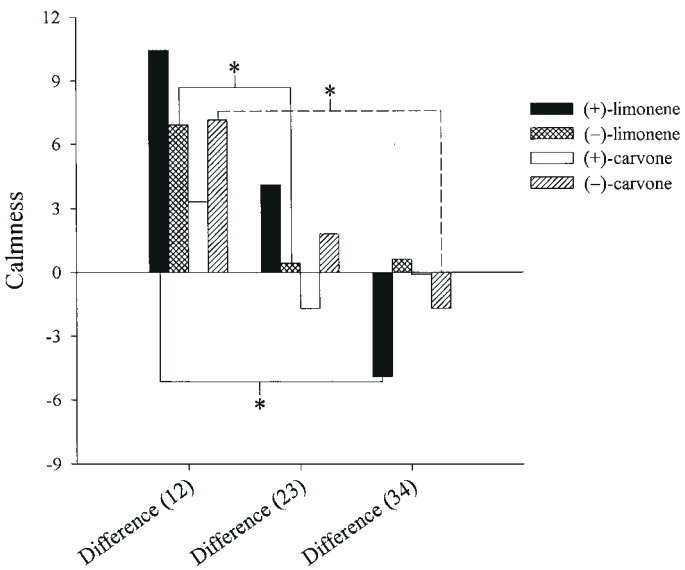

Figure 7 Mean inter-trial subjective calmness difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between trials 1 and 2; difference (23), difference score between trials 2 and 3; $*P \leq 0.05$.

In the (–)-limonene session comparison of difference scores (12) and (23) revealed a significantly larger increase in subjective calmness during the first trial than during the second ($P = 0.010$); also, comparison of difference scores (12) and (34) showed a trend towards a larger increase in subjective calmness during the first trial than during inhalation of (–)-limonene in trial 3 ($P = 0.053$). However, no significant difference was found between difference scores (23) and (34) ($P = 0.578$). In the (+)-limonene session comparison of difference scores (12) and (23) revealed a trend towards a larger increase in subjective calmness during the first trial than during the second ($P = 0.076$); comparison of difference scores (12) and (34) revealed a significant decrease in calmness during inhalation of (+)-limonene ($P = 0.020$). However, no significant difference was found between difference scores (23) and (34) ($P = 0.159$).

No significant differences were found between the limonene sessions [difference (12), $P = 0.296$; difference (23), $P = 0.192$; difference (34), $P = 0.314$].

Comparison of difference scores (12) and (34) revealed a significant decrease in subjective calmness during inhalation of (–)-carvone ($P = 0.001$). Comparison of difference scores (12) and (23) showed no significant difference ($P = 0.179$). Also, no significant difference was found between difference scores (23) and (34) ($P = 0.165$). No significant effects of (+)-carvone on subjective calmness were found [difference (12) versus difference (23), $P = 0.350$; difference (12) versus difference (34), $P = 0.499$; difference (23) versus difference (34), $P = 0.711$].

Comparison of difference scores between the carvone sessions revealed no significant differences [difference (12), $P = 0.481$; difference (23), $P = 0.459$; difference (34), $P = 0.575$].

Mood

Mean difference scores between ratings 1 and 2 [difference (12)], 2 and 3 [difference (23)] and 3 and 4 [difference (34)] of subjective mood for all sessions are presented in Figure 8. Positive difference scores between ratings 1 and 2, i.e. an increase in subjective cheerfulness during the first trial, were observed in both limonene sessions. In the (+)-limonene session positive difference scores were also found between ratings 2 and 3 (during the second trial) as well as between ratings 3 and 4 (during inhalation of the substance in the third trial). However, in the (–)-limonene session a negative difference score between ratings 2 and 3 was observed, i.e. a decrease in subjective cheerfulness during the second trial, and a positive difference score between ratings 3 and 4.

In the (–)-limonene session comparison of difference scores (12) and (23) revealed that the increase in subjective cheerfulness during the first trial was in significant contrast to the decrease during the second trial ($P = 0.016$). However, no significant effects of inhalation of (–)-limonene during the third trial were found [difference (12) versus difference (34), $P = 0.532$; difference (23) versus difference (34), $P = 0.177$]. In the (+)-limonene session comparison of difference scores (23) and (34) revealed a trend towards an increase in subjective cheerfulness during inhalation of the substance in the third trial ($P = 0.094$).

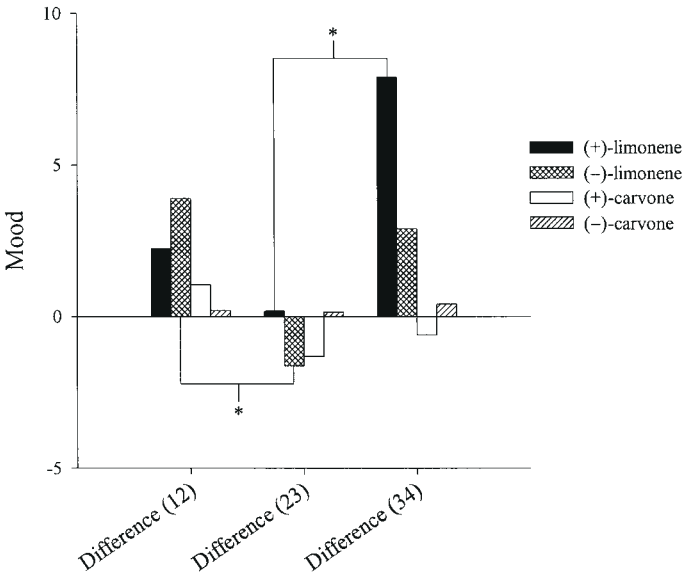

Figure 8 Mean inter-trial subjective mood difference scores for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; difference (12), difference score between trials 1 and 2; difference (23), difference score between trials 2 and 3; $^*P \leq 0.05$.

Comparison of difference scores (12), (23) and (34) between the limonene sessions showed no significant differences ($P = 0.522$, $P = 0.144$ and $P = 0.191$, respectively).

In the (–)-carvone session small positive difference scores between ratings 1 and 2 [difference (12)], 2 and 3 [difference (23)] and 3 and 4 [difference (34)] were observed. In the (+)-carvone session a positive difference score between ratings 1 and 2 [difference (12)] and negative difference scores between ratings 2 and 3 [difference (23)] as well as between ratings 3 and 4 [difference (34)] were found. Comparison of difference scores within sessions revealed no significant effects of any enantiomer of carvone on mood [(–)-carvone, difference (12) versus difference (23), $P = 0.862$, difference (12) versus difference (34), $P = 0.920$, difference (23) versus difference (34), $P = 0.165$; (+)–carvone, difference (12) versus difference (23), $P = 0.304$, difference (12) versus difference (34), $P = 0.354$, difference (23) versus difference (34), $P = 0.643$]. Also, comparison of difference scores (12), (23) and (34) between the carvone sessions revealed no significant differences ($P = 0.629$, $P = 0.489$ and $P = 0.204$, respectively).

Subjective odor rating

Figure 9 shows mean ratings of pleasantness, intensity and effect for the enantiomers of limonene and carvone. The odor of (+)-limonene was rated more pleasant ($P = 0.012$) and more stimulating ($P = 0.031$) than that of (–)-limonene but intensity ratings did not differ significantly between the

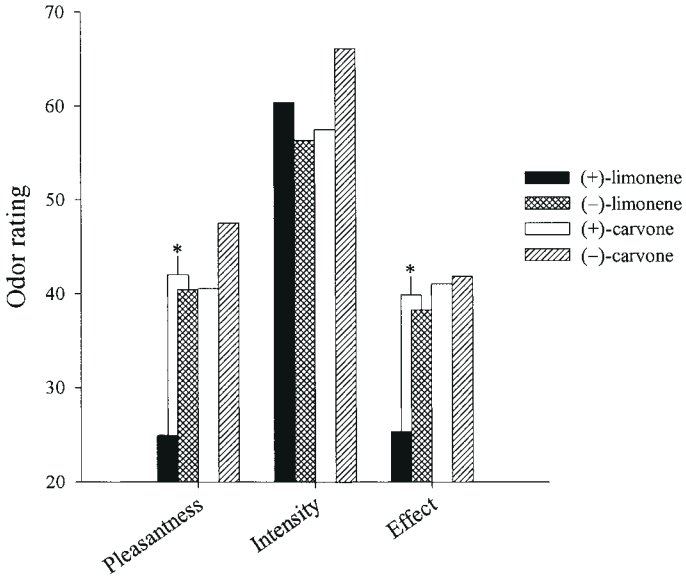

Figure 9 Mean odor rating values for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; *$P \leq 0.05$.

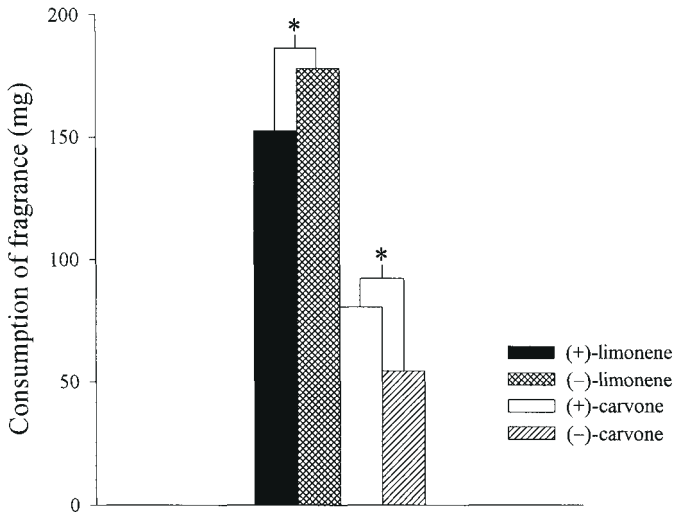

Figure 10 Mean fragrance consumption values for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone; *$P \leq 0.05$.

limonene sessions ($P = 0.305$). No significant differences between the odor ratings of (+)-carvone and (–)-carvone were found (pleasantness, $P = 0.287$; intensity, $P = 0.390$; effect, $P = 0.926$).

Consumption of fragrance

Mean values of the consumption of (–)-limonene, (+)-limonene, (–)-carvone and (+)-carvone during the third trial are presented in Figure 10. The consumption of (–)-limonene was significantly higher than that of (+)-limonene ($t = –2.857$, $P = 0.016$); the consumption of (–)-carvone was significantly lower than that of (+)-carvone ($t = –2.637$, $P = 0.01$).

Correlational analyses

For each session correlational analyses were performed on the difference scores between trials 2 and 3 of the ANS parameters, on the difference scores between ratings 3 and 4 of self-reports, on subjective fragrance ratings and on consumption of the substance. Additionally, interactions between BR in the third trial, difference scores between ratings 3 and 4 of self-reports, fragrance ratings and consumption of the substance were studied. The Spearman rank correlation coefficient (ρ) was used to analyze interactions between subjective ratings and autonomic parameters. With a sample size of $n = 20$ $\rho \geq |0.379|$ is considered to be statistically significant at the 0.05 level (Bortz, 1999). The Pearson correlation coefficient (r) was used to detect interactions among the autonomic parameters. In addition, all correlations were submitted to visual inspection in order to identify outliers which might account for statistical significance of the interaction. Significant interactions are presented in Table 1.

In the (–)-limonene session a correlation between subjective ratings of pleasantness and effect of the odor was found: the more pleasant the fragrance was rated the more stimulating it was judged ($\rho = +0.466$). Ratings of the fragrance's pleasantness and effect were correlated with changes in subjective calmness and alertness: the more pleasant the odor was judged the less calm subjects felt ($\rho = +0.495$); the more stimulating the fragrance was rated the more alert subjects felt ($\rho = –0.484$). Subjective evaluation of the fragrance's effect and intensity interacted with changes of ST and PR: the more stimulating (–)-limonene was rated the more ST decreased in the third trial ($\rho = –0.518$); the more intense the substance was rated the greater was the change in PR (increase or decrease) (U-shaped, $r = 0.653$, $P = 0.001$).

Also, in the (+)-limonene session subjective rating of the fragrance's effect was correlated with subjective rating of pleasantness: the more pleasant the fragrance was judged the more stimulating it was rated ($\rho = +0.403$). Interactions were revealed between subjective evaluation of the odor and change in subjective alertness: the more intense and the more stimulating the substance was rated the more alert subjects felt at the end of the third trial ($\rho = +0.716$ and $–0.527$, respectively). Subjective rating of the substance was also correlated with changes in BOS, non-specific fluctuations of SC and SBP: the more pleasant the odor of (+)-limonene was rated the more BOS increased during the third trial ($\rho = +0.455$); the more intense the odor was rated the more the number of non-specific fluctuations of SC increased ($\rho = –0.552$) during the third trial; the less or the more intense the substance was experienced the less SBP rose (U-shaped, $r = 0.701$, $P = 0.002$).

In the (–)-carvone session change in subjective mood was correlated with subjective evaluation of odor intensity and

Table 1 Correlational analyses for (+)-limonene, (–)-limonene, (+)-carvone and (–)-carvone

Substance	ANS/ANS	ANS/SE	ANS/FR	SF/ANS	SE/SE	SE/FR	SF/SE	FR/FR	SF/FR
(–)-Limonene			ST↓/E↑ PR/I∪			C↓/P↑ A↑/E↑		P↑/E↑	
(+)-Limonene			BOS↑/P↑ SBP/I∪ NSF↑/I↑			A↑/E↑ A↑/I↑		P↑/E↑	
(+)-Carvone	SBP↑/DBP↑	BOS↑/C↓	SBP↑/E↑ DBP↑/E↑ ST↑/E↑ ST↑/P↑ PR↑/P↑			A↑/P↑ A↑/E↑			
(–)-Carvone		BR↑/C↑ DBB↓/C↑ DBP↓/M↑ SBP↓/M↑ BR3↓/M↓	BR3↓/I↑ BR3↓/E↑	SF↑/SBP↑ SF↑/BR3↓	C↑/M↑	M↓/I↑ C↓/E↑	SF↑/M↓ SF↑/C↓		SF↑/I↑ SF↑/E↑

ANS, ANS parameter; BOS, blood oxygen saturation [difference score (23)]; BR, breathing rate [difference score (23)]; DBP, diastolic blood pressure [difference score (23)]; PR, pulse rate [difference score (23)]; SBP, systolic blood pressure [difference score (23)]; NSF, rate of non-specific fluctuations of skin conductance [difference score (23)]; ST, skin temperature [difference score (23)]; ↑/↓, increase/decrease in trial 3 as compared with trial 2; ∪, U-shaped interaction; BR3, breathing rate in trial 3; ↑/↓, increase/decrease in trial 3; SE, self-evaluation; A, alertness [difference score (34)]; C, calmness [difference score (34)]; M, mood [difference score (34)]; ↑/↓, increase/decrease on rating 4 as compared with rating 3; FR, fragrance rating; E, (stimulating) effect; I, intensity; P, pleasantness; ↑/↓, increase/decrease of rated odor quality; SF, consumption of fragrance; ↑/↓, increase/decrease.

change in subjective calmness interacted with rating of the odor's effect: the more intense the substance was judged the less cheerful subjects felt (ρ = –0.704) and the more activating the odor was rated the less calm subjects rated themselves (ρ = +0.414). Interactions were found between BR in the third trial and subjective evaluation of the fragrance's intensity and effect: the slower BR was during the third trial the more intense and the more stimulating (–)-carvone was judged (ρ = –0.554 and +0.628, respectively). Changes in subjective calmness interacted with changes in subjective mood: the more cheerful subjects felt the less restless they rated themselves (ρ = +0.608). Changes in subjective calmness and mood were correlated with changes in BR, SBP, DBP and BR in the third trial: the less calm subjects felt the more BR decreased (ρ = –0.412) and the more DBP rose during the third trial (ρ = +0.394); the more cheerful subjects felt the faster BR was during the third trial (ρ = +0.759); the more cheerful subjects felt the less SBP and DBP increased during the third trial (ρ = +0.564 and +0.381, respectively). Significant interactions were revealed between the consumption of (–)-carvone and BR in the third trial, changes in SBP, subjective mood, subjective calmness and subjective ratings of the odor: the higher the consumption of (–)-carvone during the third trial the slower was BR during this trial (r = –0.571, P = 0.017), the more SBP increased (r = –0.462, P = 0.040), the less cheerful and the less calm subjects felt (ρ = –0.454 and –0.499, respectively) and the more intense as well as stimulating the odor was rated (ρ = +0.506 and –0.439, respectively).

In the (+)-carvone session ratings of the substance's pleasantness and effect were found to interact with changes in subjective alertness: the more pleasant and the more stimulating (+)-carvone was judged the more alert subjects felt (ρ = –0.512 and –0.644, respectively). Also, ratings of the odor's pleasantness and effect interacted with changes in SBP, DBP, ST and PR: the more stimulating the fragrance was rated the more SBP as well as DBP rose (ρ = +0.473 and +0.615, respectively) and the more ST increased during the third trial (ρ = +0.479); the more pleasant the substance was judged the more ST and PR increased during the third trial (ρ = +0.698 and +0.577, respectively). Changes in subjective calmness were correlated with changes in BOS: the less calm subjects felt the more BOS increased during the third trial (ρ = +0.641). A significant correlation between changes in SBP and DBP was found: the higher the increase in SBP was the more DBP rose (r = +0.522, P = 0.026).

Discussion

For the effects of fragrances on human autonomic functioning and behavior two mechanisms are discussed: pharmacological, i.e. a direct interaction between odor molecules and receptor or nerve endings, and psychological, i.e. via the

involvement of subjective experience of odor (Jellinek, 1997).

The results of the present study indicate the involvement of both processes to varying degrees. In the (–)-carvone session decreases in PR, SBP and DBP were found between the first and second trials, which indicate a decrease in autonomic arousal. These findings are in agreement with the decrease in subjective alertness and the increase in subjective calmness which were observed, especially during the first trial. In the third trial, however, a stimulating effect of the substance was observed which can be interpreted in terms of both pharmacological and psychological mechanisms. Inhalation of the fragrance was associated with significant increases in PR and DBP as well as a significant decrease in subjective calmness. The increase in subjective alertness during the third trial is, however, not clearly attributable to (–)-carvone, since a trend towards increasing alertness had already been observed during the second trial. Correlation analyses revealed that the individual consumption of substance during the third trial interacted with the subjects' estimates of the fragrance's intensity and effect: the higher the consumption of (–)-carvone the more intense and the more activating its odor was rated. Since the air flow through the fragrance delivery system to the breathing mask was maintained at a constant rate, the variation in consumption must be related to breathing behavior, i.e. higher consumption must coincide with either an elevated BR or a higher tidal volume, which is associated with a slower BR when subjects are at rest. In fact, there was a correlation between substance consumption and BR in the third trial. Also, estimates of the odor's intensity and effect were related to BR in the third trial. It may be hypothesized that a slower BR in the third trial was accompanied by higher tidal volumes leading to higher consumption of (–)-carvone, i.e. increased intake of the substance. Since higher overall consumption of the fragrance also indicates higher concentration of the odor per breath, it was rated more intense and more activating. These interactions clearly represent a dose–effect relation. Moreover, interactions between substance consumption and changes in ANS parameters (SBP) as well as self-reports (mood and calmness) between the second and third trials were found: the higher the consumption of (–)-carvone the more SBP increased and the less cheerful and calm subjects felt. These correlations may also be interpreted in terms of a dose–effect relation and give evidence for a pharmacological factor involved in the effects of (–)-carvone.

The intensity of (–)-carvone was correlated with changes in subjective mood and the effect of the odor was related to changes in subjective calmness, i.e. subjects felt less cheerful the more intense the odor was judged and they felt less calm the more activating the odor was rated. Changes in subjective calmness and mood were correlated with changes in DBP: the less cheerful and less calm subjects felt the more DBP increased. These findings rather point to an individual/psychological process initiated by subjective experience of the odor of (–)-carvone than to a direct pharmacological effect of the substance.

However, the increase in PR was neither correlated with fragrance ratings nor with changes in self-evaluation, which again suggests a pharmacological mechanism for this effect of (–)-carvone.

In the (+)-carvone session a decrease in autonomic arousal between the first and second trials was observed in terms of decreases in PR, SBP and DBP. Subjective ratings of alertness, calmness and mood only marginally changed during these trials. During inhalation of (+)-carvone significant increases in SBP as well as DBP and a trend towards a smaller decrease in PR were observed, whereas subjective measures remained unchanged. These findings can quite convincingly be explained by the correlations of fragrance ratings with self-evaluation and ANS parameters and may be interpreted in terms of an activation mediated by subjective experience of the odor. Subjects felt more alert the more pleasant and activating the fragrance was judged. PR increased the more pleasant the fragrance was rated. SBP as well as DBP increased the more stimulating subjects rated the odor of (+)-carvone.

In both limonene sessions decreases in PR and SBP between the first and second trials were found, indicating a decrease in autonomic arousal. With respect to DBP a decrease between the first and second trials was observed in the (+)-limonene session, whereas an increase was found in the (–)-limonene session. However, comparison of these difference scores between sessions failed to reach statistical significance. In both limonene sessions subjectively experienced calmness increased during the baseline trials, confirming the observed decrease in autonomic arousal. With respect to subjective alertness and mood the situation is not so clear. In both limonene sessions subjective alertness decreased during the first trial. However, in the (–)-limonene session subjects felt significantly less alert after the first trial than in the (+)-limonene session. In both limonene sessions subjective alertness increased during the second trial. However, only in the (–)-limonene session did subjects feel significantly more alert after this trial. Also, in both limonene sessions subjects' cheerfulness increased during the first trial. In the (+)-limonene session mood remained nearly unchanged during the second trial, whereas it decreased significantly in the (–)-limonene session. The significant changes in subjective alertness and mood during the second trial seem to agree with the increase in DBP between the first and second trials which was observed in this session. However, no relationships were found between these measures. Inhalation of (+)-limonene led to significant increases in SBP and subjective alertness as well as a significant decrease in subjective calmness. In addition, a trend towards an increase in subjective cheerfulness was observed. Inhalation of (–)-limonene led to a significant increase in SBP. Also, subjects reported feeling significantly more alert

after the third trial. However, this finding is unlikely to be due to inhalation of the fragrance since a significant increase in subjective alertness had already occurred during the second trial. Ratings of subjective calmness were not affected by administration of the odor; the increase in subjective cheerfulness during the third trial failed to reach statistical significance. Similarly to the (+)-carvone session, the physiological and behavioral arousal which was determined during inhalation of the enantiomers of limonene is in agreement with the interactions between ratings of the fragrances and self-evaluation. In both sessions relationships between the substance's pleasantness and effect and subjective alertness were revealed. The more stimulating the fragrances were judged the more pleasant they were rated and the more alert subjects felt. In addition, in the (+)-limonene session intensity of the odor interacted with subjective alertness, i.e. the more intense the fragrance was rated the more alert subjects felt. However, a U-shaped relationship was revealed between subjective ratings of the fragrance's intensity and SBP. The increase in SBP was highest when the odor intensity was perceived as intermediate. In other words, higher subjective intensity estimates did not result in further increases in individual physiological arousal. This may indicate that, on the physiological level, activation is limited and that increasing the strength of the stimulus cannot overcome this limit (Putnam and Vanman, 1999). In the (–)-limonene session correlations between odor pleasantness, subjective calmness and mood and SBP were found. The more pleasant the substance was judged the less calm and the more cheerful subjects rated themselves; the more cheerful subjects felt the more SBP increased during inhalation.

Comparison of the limonene sessions showed that the consumption of (+)-limonene was significantly lower than that of (–)-limonene. Although there were no significant differences between these sessions with respect to ANS parameters and self-evaluation, it is evident that inhalation of (+)-limonene led to increases in both physiological and behavioral arousal, whereas administration of (–)-limonene affected ANS parameters only. On the other hand, the odor of (+)-limonene was rated significantly more pleasant and more activating than that of (–)-limonene. A significant difference in consumption was also found between the enantiomers of carvone, that of the (+)-enantiomer being significantly higher than that of the (–)-enantiomer. With respect to the measures of arousal, there appears to exist a similar pattern as in the limonene sessions: (+)-carvone affected autonomic arousal only, whereas (–)-carvone caused increases in both physiological and behavioral arousal. However, subjective ratings of the odor did not differ between the antipodes. Especially in the case of the limonene sessions, these findings suggest a relationship between subjective evaluation of an odor and the observed effects on arousal and mood which have been emphasized, for example, by Lorig and Roberts (1990) and Knasko (1992).

Elucidation of the different mechanisms as stated above is crucial to our understanding of the effects of odors on human bodily functions and on behavior. As the current investigation has shown, several mechanisms are likely to be involved when fragrances are administered by means of inhalation. Moreover, they seem to be effective simultaneously. In order to investigate pharmacological effects separately, subjective evaluation of odors must be prevented. As experiments recently conducted by Buchbauer's group (Heuberger *et al.*, 1999) have shown, pharmacological effects are accessible by means of topical administration of substances and exclusion of olfactory processing.

In conclusion, we report that prolonged inhalation of fragrances leads to alterations of both physiological data and subjective mental and emotional condition. The effects of fragrances are to a considerable extent mediated by subjective experience of an odor. Some evidence was found that chirality of the odorants exerts an influence on their mode of action, indicating that, in analogy with other pharmaceuticals, the antipodes may behave differently.

Acknowledgements

The authors are grateful to the Austrian National Bank Jubilee Fund (project no. 6718; grant to E.H.), to a Sirindhorn Scholarship (Srinakharinwirot University, Bangkok, Thailand; grant to T.H.) and to Dragoco GmbH (Vienna) for interest in this study.

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Accepted November 21, 2000