Adv. Hort. Sci., 2014 28(2): 90-94

Physiological and psychological effects of olfactory stimulation with D-Limonene

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Key words: heart rate, heart rate variability, limonene, physiological relaxation, semantic differential method.

Abstract: Although D-Limonene can be considered an important component of nature-based stimuli, the physiological effects of olfactory stimulation with D-Limonene have not been completely clarified by scientific studies. The physiological and psychological effects of olfactory stimulation with D-Limonene were studied measuring heart rate variability (HRV), heart rate, and subjective evaluation using a modified semantic differential method; thirteen Japanese female university students (mean age±SD, 21.5±1.0 years) participated in the study. A concentration of 60 µL of D-Limonene was used as olfactory stimulant and room air as control. Subjects were exposed for 90 s while sitting with eyes closed. During D-Limonene inhalation: (1) the high-frequency (HF) value of HRV, a marker of parasympathetic nervous activity that is enhanced in relaxing situations, was significantly higher; (2) the heart rate was significantly lower; and (3) subjects reported feeling significantly more comfortable during D-Limonene administration than control. The results obtained clearly indicate that olfactory stimulation with D-Limonene induced physiological and psychological relaxation, providing important scientific evidence of the health benefits of D-Limonene.

1. Introduction

In the modern age, people are forced to lead busy lives and are exposed to a state of stress (Lederbogen *et al.*, 2011). Thus, measures to prevent and relieve this stress state are urgently needed.

Recently, forest therapy has emerged as a method to address stress states, and much data on the physiological and psychological relaxing effects of forest environments have been accumulated. Previous studies have reported that viewing forest scenery or walking in forests can: increase parasympathetic nervous activity, which is enhanced in relaxing situations and suppresses sympathetic nervous activity which is increased in stress states (Tsunetsugu *et al.*, 2007; Park *et al.*, 2008; Lee *et al.*, 2009; Park *et al.*, 2009; Park *et al.*, 2010; Lee *et al.*, 2011; Park *et al.*, 2012; Tsunetsugu *et al.*, 2013; Lee *et al.*, 2014); decrease cerebral blood flow in the prefrontal cortex (Park *et al.*, 2007); and decrease salivary cortisol concentration of stress hormone (Tsunetsugu *et al.*, 2007; Park *et al.*, 2007; Park *et al.*, 2008; Lee *et al.*, 2009; Park *et al.*, 2010). In addition, visiting a forest enhanced natural killer-cell activity and improved immune function (Li *et al.*, 2007; Li *et al.*, 2008 a, b, c) and the effect lasted 30 days (Li *et al.*, 2008 b). In subjective evaluations, it was reported that people feel more "comfortable," "soothed," and "natural" when experiencing a forest environment (Park *et al.*, 2007; Tsunetsugu *et al.*, 2007; Park *et al.*, 2008; Lee *et al.*, 2009; Park *et al.*, 2009; Lee *et al.*, 2011; Park *et al.*, 2011; Tsunetsugu *et al.*, 2013; Lee *et al.*, 2014), and that the "tension-anxiety," "depression," "anger-hostility," "fatigue," "confusion," and "vigor" of the mood state profile (McNair and Lorr, 1964; McNair *et al.*, 1992; Yokoyama, 2005) improved (Li *et al.*, 2008 a, b, c; Park *et al.*, 2010; Lee *et al.*, 2011; Park *et al.*, 2011; Tsunetsugu *et al.*, 2013; Lee *et al.*, 2014). Unfortunately, many people living in cities find it difficult to access forest environments. Thus, much attention has been focused on nature-based stimuli, such as walking in an urban park (Song *et al.*, 2013), viewing rooftop forests (Matsunaga *et al.*, 2011), the presence of plants, including dracaena (Igarashi *et al.*, 2014) or roses (Ikei *et al.*, 2014), and physical contact with wood (Sakuragawa *et al.*, 2008), and the relaxing effects of these stimuli have been reported.

Nature-based stimuli are intuitively perceived through the five senses. Of these five senses, the physiological effects of olfactory stimulation have been characterized

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Received for publication 31 March 2014
Accepted for publication 30 June 2014

in detail. Miyazaki *et al*. (1992) conducted a pioneering study which revealed that olfactory stimulation with *Chamaecyparis taiwanensis* essential oil significantly decreased blood pressure. Furthermore, inhalation of rose oil odor was shown to suppress sympathetic nervous activity and decrease adrenaline concentration (Haze *et al*., 2002). Lavender oil has been shown to induce deep sleep (Goel *et al*., 2005) and improve concentration (Sakamoto *et al*., 2005).

However, evidence-based research using the indices of autonomic nervous activity to clarify the effect of components of these essential oils is lacking.

The essential oil components of *Cryptomeria japonica* and *Pinus densiflora*, representative forest trees, have been reported (Cimanga *et al*., 2002; Hong *et al*., 2004; Cheng *et al*., 2009). These oils are composed of various volatile organic compounds, including D-Limonene, α-Pinene, β-Pinene. D-Limonene is the main component of citrus peel oil (Bernhard, 1960; Attaway *et al*., 1968; Shaw, 1979; Chiralts *et al*., 2002; Yoo *et al*., 2004).

The purpose of the present study was to investigate the physiological effect of olfactory stimulation with D-Limonene on autonomic nervous activity by measuring its effect on heart rate variability (HRV) (Camm *et al*., 1996; Kobayashi *et al*., 1999) and the heart rate.

2. Materials and Methods

Subjects

Thirteen Japanese female university students (age range, 21.5±1.0 years; mean±SD) participated in the study. Before beginning the experiment, a full explanation about the research aim, the experimental procedure, and all measured indices was provided. Informed consent was obtained from all subjects. This study was conducted in accordance with the regulations of the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan.

Study protocol

Physiological and psychological measurements were carried out in a chamber with an artificial climate maintained at 25°C with 50% relative humidity and 230-lux illumination. D-Limonene (>95.0% purity, Tokyo Chemical Industry Co., Ltd., Japan) was used as an olfactory stimulant, and room air was used as a control. A total of 60 μL D-Limonene was injected into a 24-L odor bag (polyethylene terephthalate film heat seal bag; NS-KOEN Co., Ltd., Kyoto, Japan) and the odors were presented to each subject by means of a device fitted on the chest and situated approximately 10 cm under the nose (Fig. 1). The flow rate of the odor was set at 3 L/min. Subjective sensitivity to the odor was determined in a preliminary investigation. The subjects were exposed to the odor for 90 s while sitting with their eyes closed. The order of presentation of D-Limonene and control was counterbalanced.

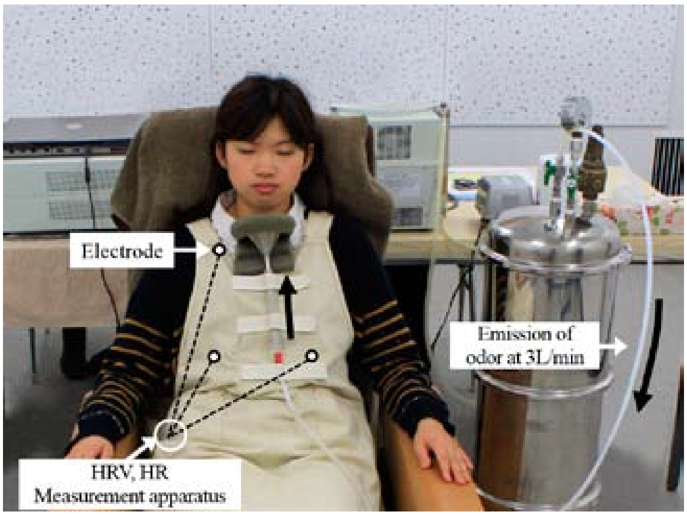

Fig. 1 - Olfactory stimulation setup.

Heart rate variability and heart rate

HRV was measured as the periods between consecutive R waves (R-R intervals) in an electrocardiogram recorded with a portable electrocardiograph (Activtracer AC-301A, GMS, Japan). In this study, two major spectral components of HRV, the low-frequency (LF; 0.04–0.15 Hz) band and the high-frequency (HF; 0.15-0.40 Hz) band were obtained by the maximum-entropy method (MemCalc/Win, GMS, Japan). The HF power was considered to reflect parasympathetic nervous activity, and the LF/HF power ratio was considered to reflect the sympathetic nervous activity (Camm *et al*., 1996; Kobayashi *et al*., 1999). Heart rate was also investigated using R-R interval data.

Semantic differential method

The subjects provided a subjective evaluation of the emotional impact of the odors according to a modified semantic differential (SD) method (Osgood *et al*., 1957). This method allowed the subject to assess a pair of adjectives, such as "comfortable-uncomfortable," using a 13-point scale. The SD method was performed after administration of each odor.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences software version 20.0 (IBM Corp., Armonk, NY, USA). A paired t-test was used to compare differences in the physiological responses over the 90 s of exposure to D-Limonene and air. Wilcoxon signed-rank test was applied to analyze differences in psychological response between D-Limonene and air. A one-sided test was used in this study. In all cases, the significance level was set at $P < 0.05$.

3. Results

The results of the HRV data after exposure to D-Limonene and control were compared, and a significant difference was found in the HF value, which is a marker of parasympathetic nervous activity, as shown in Figure 2. The HF value increased 26.4% during D-Limonene administration (827.2±191.3 ms^2; mean±SE) compared with control (654.4 ±163.6 ms^2), indicating that parasympathetic nervous activity was significantly higher during D-Limonene administration (P<0.05). However, no significant difference was found in the LF/HF power ratio for the two stimuli.

Figure 3 shows a comparison of the heart rate during the administration of D-Limonene and control. Heart rate decreased during D-Limonene administration (72.8±2.3 bpm) compared with control (74.1±2.5 bpm), and this difference was significant (P<0.05).

Figure 4 shows the results for a "comfortable" feeling according to the subjective evaluation. Subjects reported significantly more comfortable ratings during D-Limonene administration than control (P<0.01).

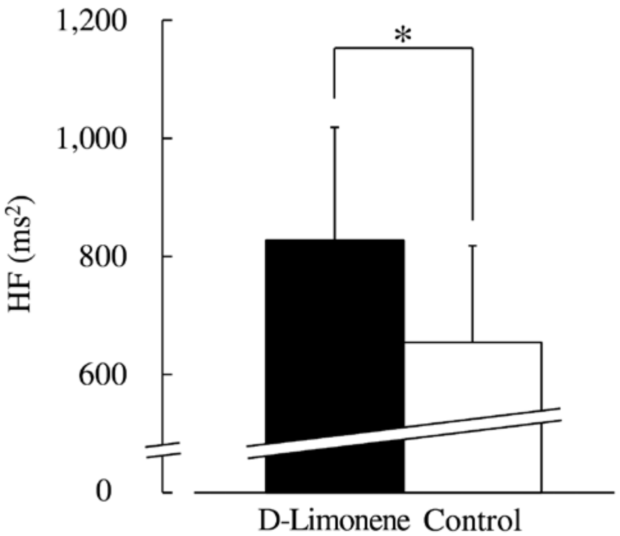

Fig. 2 - Comparison of high-frequency power levels of heart rate variability during olfactory stimulation with D-Limonene or control (air). Data are expressed as mean ± SE; n = 13. *P < 0.05 by paired t-test.

4. Discussion and Conclusions

D-Limonene is one of the most common volatile organic compounds in nature (Sun, 2007). It is a major component of various citrus oils, such as lemon, orange, grapefruit, and lime (Attaway *et al.*, 1968; Bernhard, 1960; Chiralts *et al.*, 2002; Shaw, 1979; Yoo *et al.*, 2004), as well as essential oils from coniferous trees, such as *Pinus densiflora*, *Pinus koraiensis*, *Chamaecyparis obtusa*, and *Cryptomeria japonica* (Cimanga *et al.*, 2002; Hong *et al.*, 2004; Cheng *et al.*, 2009). In addition, because of its citrus fragrance, D-Limonene is commonly added to perfumes, soaps, and cosmetics (Bakkali *et al.*, 2008).

Although D-Limonene is an important component of nature-based stimuli, the physiological effect of olfactory stimulation with D-Limonene has not been completely clarified. Previously, Tsunetsugu *et al.* (2012) investigated the physiological effect of olfactory simulation with D-Limonene on blood pressure and showed that olfactory simulation with a concentration of 10 μL D-Limonene decreases subjects' systolic blood pressure. However, to our knowledge, no previous study has examined the physiological effect of olfactory stimulation with D-Limonene on HRV and heart rate.

The present study shows that olfactory stimulation with D-Limonene induced (1) a significant increase in parasympathetic nervous activities, (2) a significant decrease in the

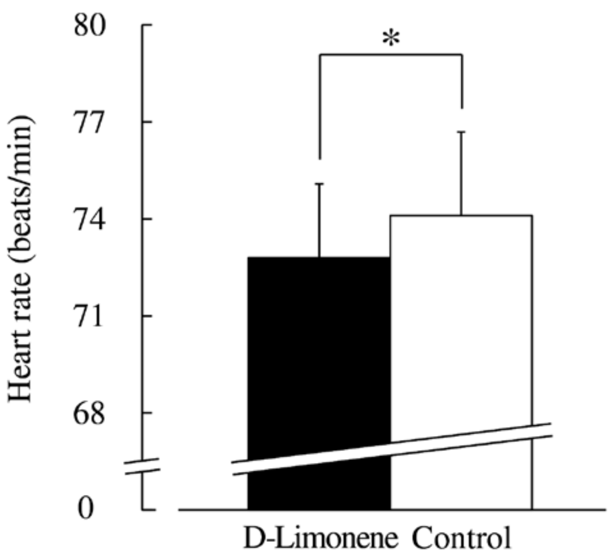

Fig. 3 - Comparison of the heart rate during olfactory stimulation with D-Limonene or control (air). Data are expressed as mean ± SE; n = 13. *P < 0.05 by paired t-test.

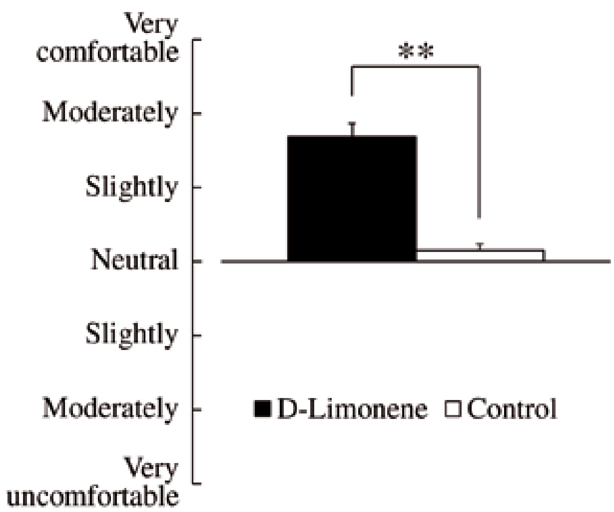

Fig. 4 - Subjective evaluation of "comfortable" measured by a modified semantic differential questionnaire after olfactory stimulation with D-Limonene or control (air). Data are expressed as mean ± SE; n = 13. **P < 0.01 by Wilcoxon signed-rank test.

heart rate, and (3) a significant increase in a "comfortable" feeling. These results agree with previous studies of other nature-based stimuli (Tsunetsugu *et al*., 2007; Park *et al*., 2008; Park *et al*., 2009; Park *et al*., 2010; Lee *et al*., 2011; Park *et al*., 2012; Song *et al*., 2013; Tsunetsugu *et al*., 2013; Ikei *et al*., 2014, Lee *et al*., 2014). Park *et al*. (2012) showed that the HF value of HRV was significantly increased while viewing scenery of forests using the results of field experiments at 35 forests in Japan. Ikei *et al*. (2014) reported that the HF component was significantly increased by viewing roses. Song *et al*. (2013) revealed that parasympathetic nervous activity was enhanced and the heart rate was significantly lower after walking in an urban park than walking in a city area. Our results support the hypothesis that olfactory stimulation with D-Limonene has a relaxation effect that is similar to other nature-based stimuli.

In conclusion, our results clearly indicate that olfactory simulation with D-Limonene induced physiological and psychological relaxation. And these finding provide important scientific evidence on the health benefits of D-Limonene exposure.

As all the participants in this study were healthy females in their twenties, further studies are needed to ascertain the effect in diverse groups, including males and different age groups. In addition, it is necessary to examine the effect using multiple indices, such as prefrontal cortex activity, stress hormones, and others.

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