The effects of plant-derived stimulation on the physiological response

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植物由来の刺激が生理応答に及ぼす影響

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Contents

1. Introduction

The “relaxation effect on humans” plays an important role in the field of horticulture. Societal progression over the past few centuries has resulted in increasingly stressful experiences for humans because of artificial environments. Various actions may reduce stress, a focus that is suddenly increasing throughout modern-day societies. Accordingly, familiar and convenient stimulations derived from nature are actively adapted with the expectations that they provide relaxation. The effects of plants, in particular, have received considerable attention because of their relaxation effects on the five senses. Empirical evidence shows that plant-derived stimulations deliver a relaxation effect, but scientific evidence regarding this is insufficient.

To comprehend the recent data accumulation of the physiological effects of plant-derived stimulation, we performed a research on the daily use of perilla for Kampo. Perilla is a type of aromatic vegetable. Asian people preferred consuming it since ancient times, and perilla is used as a traditional medicine and functional food in Asia.In traditional medicine, aromatic substances are often used to treat mental stress. Perilla is included as one of them, and it is also used in combination with other aromatic Oriental medicines that are referred to as Kampo medicines (hereafter ‘Kampo’) [1, 2, 3, 4]. However, the importance and role of perilla in these Kampo medicines remain to be clarified. In Japan, these Kampo have been used as medicine for a long time and are now approved and used as general medicines. In recent years, Kampo has attracted attention as an alternative medicine among the foreign countries, including those in Europe and the United States, and the World Health Organization (WHO) announced that they will add a chapter on traditional medicines, including Japanese Kampo, in the eleventh edition of the International Classification of Diseases (ICD-11) in 2015. Accordingly, scientific investigations of the effects of Kampo are advancing [5, 6, 7, 8, 9, 10]. Nonetheless, for many years Kampo has been used in traditional Asian medicines to treat physical conditions. We review the use of bioactive compounds from perilla varieties as medicines and foods to prevent and ameliorate illnesses.

Next, we conducted an indoor experiment to evaluate the independent effects of plant-derived stimulations and studied the physiological effects of perilla, rose, and orange essential oils, and fresh rose flowers for evaluating olfactory stimulations.

With respect to perilla, little is known on the effects of these Kampo in humans and on the role of perilla. Most Kampo have been used as medicines for a long time and have been approved for use as general medicines, including perilla, which does not have any status as a medical drug with proven efficacy, but has been approved as an herbal medicine. Perillaldehyde, which is the primary component of perilla, has been reported to have an antidepressant-like effect via the olfactory nervous system *in vivo* in a murine model [11]. However, till date, the physiological effects of olfactory stimulation with perilla in humans have not yet been identified [12].Given the tendency toward an increase in the number of individuals suffering from stress-related diseases worldwide [13, 14],determining the physiological effects of perilla and its ability to induce relaxation is important.We had determined previously the physiological effects of nature on human physiology and quality of life [15-20] and that near-infrared time-resolved spectroscopy is a useful technique for performing such studies [21-23]. Measurement of the prefrontal cortex activity is possible at 1 Hz because the change in oxyhemoglobin (oxy-Hb) concentration can be measured using this method. Accordingly, the aim of the present study was to determine the effects of olfactory stimulation with perilla on the human prefrontal cortex activity.

Next, we focused on rose and orange essential oils, used throughout the ages, and performed olfactory-stimulation experiments. One of the most often used fresh flowers throughout Japan is the chrysanthemum; however, we chose the rose, which is the second most popular flower after the chrysanthemum, because the chrysanthemum is known as the Buddha flower in Japan. We found very little physiological data on either rose or orange essential oils in olfactory stimulations. On the other hand, essential oils are easy to use for olfactory stimulations. The process of extracting essential oils adds heat by steam distillation, presenting volatilization at low boiling points. Therefore, we performed olfactory-stimulation experiments using actual fresh rose flowers to resolve this problem.

Finally, we compared a display image with actual plants and then examined a three-dimensional (3D) image for visual-stimulation experiments. Imaging techniques provide an opportunity to develop a realistic image, with familiar and convenient display images reflecting a plant of interest. No comparative studies have used large two-dimensional (2D) display images and actual plant physiological data. Hence, we studied the differences between actual foliage plants and display image stimulations using familiar foliage plants commonly that are used in offices and homes. While 3D images have recently attracted attention due to their high interpretations as real, there were no physiological relaxation effects from 3D plant image stimulations. The aim of this study was to compare physiological responses to various 3D visual image stimulations of plants and then compare those responses with 2D images.

The purpose of this paper is to elucidate the physiological effects of plant-derived stimulations, empirically known from the viewpoint of evidence-based medicine (EBM) and for which accumulation of scientific data will contribute to quality of life (QOL) improvement while living under artificial environments.

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2. A review on bioactivities of perilla: progresses in research on the functions of perilla as medicine and food

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Abstract:

Perilla is a useful pharmaceutical and food product and is empirically consumed by humans. However, its properties have not been evaluated extensively. In this review, we summarize the progress made in research, focusing on the bioactivities of perilla. There are many *in vitro* and animal studies on the cytostatic activity and antiallergic effects, respectively, of perilla and its constituents. However, its influence on humans remains unclear. Hence, investigating and clarifying the physiological effects of perilla and its constituents on humans are imperative in the future to adhere to the ideals of evidence-based medicine.

2.1. Introduction

Perilla is a type of aromatic vegetable. Asian people preferred consuming it since ancient times. It was used to treat fish and crab poisoning symptoms according to Chinese classics [1]. This information passed on from China throughout Asia; thereafter, perilla is used as a traditional medicine and functional food in Asia.

In traditional medicine, aromatic substances are often used to treat mental stress. Perilla is included as one of them, and it is also used in combination with other aromatic Oriental medicines that are referred to as Kampo medicines (hereafter 'Kampo'). Hangekobokuto (Chinese name: Banxia-houpo-tang), Kososan (Chinese name: Xiang-Su-San) and Suyu-Jiaonang (SYJN) are representative Kampo, and these are used to treat depression-related diseases and asthma [2]. It is reported that Hangekobokuto can ameliorate sleep choking syndrome [3], swallowing reflex [4, 5] and panic disorder [6] in humans and has antidepressant effects in mice [7]. Ito et al [8] reported that Kososan exhibited antidepressant-like effects in murine forced swimming model, and Suyu-Jiaonang (SYJN) demonstrated anti-stress effects in rats [9]. However, the importance and role of perilla in these Kampo medicines remain to be clarified.

In Japan, these Kampo have been used as medicine for a long time and are now approved and used as general medicines. In recent years, Kampo has attracted attention as an alternative medicine among the foreign countries, including those in Europe and the United States, and the World Health Organization (WHO) announced that they will add a chapter on traditional medicines, including Japanese Kampo, in the eleventh edition of the International Classification of Diseases (ICD-11) in 2015. Accordingly, scientific investigations of the effects of Kampo are advancing [4, 5, 6, 7, 8, 9]. Nonetheless, for many years Kampo has been used in traditional Asian medicines to treat physical conditions such as stress and asthma.

We review the use of bioactive compounds from perilla varieties as medicines and foods to prevent and ameliorate illnesses.

2.2. Methodology

All published reports on physiological functions of perilla were extracted from the PubMed database.

2.3. Perilla as food and Kampo

Two types of perilla, *Perilla frutescens* (L.) Britton var. *crispa* (Thunb.) H. Deane f. *purpurea* (Makino) Makino and *Perilla frutescens* (L.) Britton var. *crispa* (Thunb.) H. Deane *viridi-crispa*) are commonly added to food in Japan. In addition, perilla oil is extracted from *Perilla ocymoides* (*Perilla frutescens* (L.) Britton var. *frutescens*) for consumption in Asia [10] (Table 1).

In Japanese pharmacy, leaves and branches of *Perilla frutescence* Britt. var. *acuta* Kudo and *Perilla frutescence* Britt. var. *crispa* Decaisne as Perillae Herba [11] (Table 1) are defined as medicines. Table 2 shows the main constituents of Perillae Herba [11].

2.4. Bioactivity of perilla

2.4.1. Effects of the Kampo Perillae Herba

Perillae Herba has been used as an Oriental medicine for many years in Asia and has been passed on through generations by experience. In Japan, evident effects of Kampo containing perillae herba have been recently reported. However, the effects of the Perillae Herba itself are not stated clearly. Perillae Herba is included in Japanese Kampo medicines such as Hangekobokuto (Chinese name: Banxia Houpu; [3,4,5,6,12,13,14,15]), Kososan (Chinese name: Xiang-Su-San;[8]) and Saiboku-to (Chinese name: Chai-pu-tang) for the treatment of cough and stress symptoms [2]. Perillae herba is also found in Chinese Suyu-Jiaonang (SYJN), which is also used to treat mental conditions [9, 16].

2.4.2. Effects of perilla decoctions

Antiallergic effects of perilla decoctions have been demonstrated on mice. In these studies, perilla decoctions partly controlled IgA nephropathy [17] and type-I allergies [18]. It is thought that these effects are caused by rosmarinic acid. In addition, perilla decoctions demonstrated suppressive effects on mesangioproliferative glomerulonephritis in rats [18].

In HIGA mouse model (IgA renal damage), perilla decoctions alleviated IgA nephropathy through adjustments of the mucous membrane. Moreover, rosmarinic acid, present in high quantities in perilla, was found to be a constituent of perilla decoctions, and it is believed that the maximum effect of perilla decoction is caused by rosmarinic acid [17].

The effects of rabbit antirat thymocyte serum were examined in a BALB/c mouse model of mesangioproliferative glomerulonephritis, and suppressive effects were observed [19].

The positive effects of perilla decoctions were observed in ddY mice with type-I allergies [18]. After the oral administration of 500 mg/kg perilla decoction and the relative amount of rosmarinic acid in a mouse model with ear passive cutaneous anaphylaxis (PCA), allergic reactions were inhibited. Because the inhibition rates of perilla decoctions and rosmarinic acid were approximately equal, it was believed that the effect of perilla decoction depended on rosmarinic acid [18,20]. However, comparisons of the effects of rosmarinic acid with perilla decoctions indicate additional therapeutic constituents in perilla.

2.4.3. Effects of perilla extract

In cultured murine vascular smooth muscle cells, perilla extract has been shown to induce NO production and, indicating that perilla may be useful for the prevention of vascular diseases such as arteriosclerosis [21]. In addition, perilla extract increased restraint and induced cell death in human hepatoma HepG2 cells [22] . Moreover, flow cytometry and DNA microarray experiments revealed significant apoptosis and time-dependent regulation of apoptotic genes, respectively, in cells treated with perilla leaf extracts.

However, in this study the experiment was conducted *in vitro* with a high dose of the perilla extract, therefore it is unclear if perilla extract is effective *in vivo*.

2.4.4. Effects of perilla oil

Perilla oil contains large quantities of alpha-linolenic acid, an essential fatty acid, which decreases the risk of cardiovascular diseases [23]. Several *in vitro*, animal, and human nutritional studies have focused on the effects of perilla oil and have compared it with those of other oils. Perilla oil increases glucose-6-phosphatase activity, improves membrane stability [24], lowers plasma triacylglycerol levels [25], and controls liver fatty acid composition [26].

Perilla oil, which is rich in n-3 polyunsaturated fatty acids, regulates brown and white adipose tissue metabolism in a manner different from that of safflower and fish oils and contributes to physiological activities that prevent body fat accumulation, regulate glucose metabolism in rats [27], and control serum lipid concentrations [28]. Perilla oil rich in alpha-linolenic acid is effectively desaturated and elongated to form eicosapentanoic acid (EPA) and docosahexanoic acid (DHA; [29]). In addition, in light- and dark-discrimination learning tests, perilla oil-administrated senescence-accelerated mice (SAMP8) group exhibited higher discriminability than safflower oil-administrated group [30]. Moreover, serum lipids in the perilla oil-administrated SAMP8 group had a significantly greater ratio of apolipoprotein A-I (ApoA-I) to ApoA-II than those in the safflower-oil administered group [31]. Perilla oil was not as effective as soybean oil in preventing minor recurrent aphthous stomatitis; however, *in vivo*, *in vitro*, and lifestyle studies report positive effects of perilla oil on minor recurrent aphthous stomatitis [32].

2.5. Effects of perilla constituents

Perilla contains several essential oils, including (−)-perillaldehyde, (−)-perillylalcohol, (+)-limonene, alpha-pinene, and trans-shisool. Moreover, perilla contains the purple pigments shisonin and cyanin. Other constituents of perilla include rosmarinic acid, adenine, arginine, etc. [11].

2.5.1. Effects of perillaldehyde

In contrast with the Kampo cinnamaldehyde, perillaldehyde elicited antidepressant -like effects on the olfactory nervous system in mice that were subjected to chronic weak stress and a forced swimming test [33]. Antidepressant-like effects were evaluated by measuring the duration of immobility in the forced swimming test. Decreased duration of immobility was observed after 9 days of perillaldehyde inhalation. In contrast, the odorant cinnamaldehyde from cinnamon bark failed to decrease the duration of immobility. This antidepressant effect of perillaldehyde was abolished in mice induced with anosmia by zinc sulfate, confirming the olfactory mode of action. In Wistar rat aortas, vasodilatation following inhibition of Ca channels was ameliorated by perillaldehyde, with dose-dependent aorta extensibility, and relaxation with 0.01–1 mM treatments with prostaglandin F2α or norepinephrine [34]. In addition, these effects were unchanged by treatment with NG-nitro-L-arginine methyl ester or removal of aorta endothelium. Therefore, it was suggested that perillaldehyde directly affects vascular smooth muscle. Furthermore, vasodilatation was not inhibited by the β-adrenergic-receptor blocker propranolol, the phosphodiesterase inhibitor theophylline, the delayed rectifier K^+ channel blocker tetraethylammonium chloride or by the ATP-sensitive K^+ channel blocker glibenclamide. In contrast, perillaldehyde caused vasodilatation of contracted aortas via a mechanism involving Ca^{2+} transport out of the cell. Perillaldehyde caused slight vasodilatation of Ca^{2+}-ionophore (A23187) contracted aortas, but inhibited highly concentrated K^{+}-mediated vasodilatation. Given the predominance of voltage-dependent Ca^{2+} efflux, it was suggested that perillaldehyde acted as a Ca^{2+} channel inhibitior for vasodilatation.

In cultured human liver microsomes, perillaldehyde slightly inhibited the hydroxylation of bupropion (antidepressant) by the enzyme CYP2B6 [35] and inhibited *Candida albicans* [36]. In addition, in 293 TRPA1-HEK cells, perillaldehyde activated the transient potential A1 receptor [37], suggesting that this discovery will enable future analysis of taste properties.

Perillaldehyde inhibited the proliferation of human squamous cell carcinoma of the tongue (BroTo) and human lung adenocarcinoma (A549) [38] and demonstrated anticancer activity in PC12 cells (rat pheochromocytoma cell line [39]). Moreover, Masutani et al. [40] reported the activation of the Nrf2-Keap1 system by perillaldehyde. Furthermore, antibacterial actions (using air washers) against floating microbes were reported [41].

Perillaldehyde was found to preserve fruits and promote the antioxidant activity of blueberries [42] and Chinese bayberries [43]. Hence, these natural products may be helpful in the maintenance of crop quality and security. However, further research is required to examine the influence of perillaldehyde on flavour, texture, and harvest parameters of crops.

2.5.2. Perillyl alcohol

Perillyl alcohol is the subject of only few *in vitro* studies. In these studies, perillyl alcohol induced cell cycle arrest and cell death in BroTo; [38], and A549; [38]. Perillyl alcohol also had inhibitory effects on HCT116 cells (human colon cancer cell line; [44]) and caused dose-dependent inhibition of mammary tumor cell proliferation.

2.5.3. Perillic acid

Perillic acid has been shown to inhibit proliferation of HCT116 cells (human colon cancer; [44]), mammary tumor cells [45], and PC12 cells (rat pheochromocytoma; [39]), indicating anticancer properties.

2.5.4. Rosmarinic acid

Rosmarinic acid is a constituent of rosemary; therefore, there are several reports on this constituent of perilla.

It was reported that rosmarinic acid inhibits seasonal allergic rhinoconjuctivitis in humans [46]. This double-blind study indicated that oral supplementation with rosmarinic acid is an effective intervention for patients in the age group of 21–53 years with mild seasonal allergic rhinoconjuctivitis. In this 21-day experiment, rosmarinic acid significantly increased responder rates for itchy nose, watery eyes, itchy eyes, and total symptoms and decreased neutrophil and eosinophil numbers in nasal lavage fluid. The authors suggest that rosmarinic acid may reduce treatment costs for allergic diseases.

The absorption, metabolism, and urinary excretion of rosmarinic acid after a single dose of perilla extract was determined in six healthy men (mean age 37.2 ± 6.2 years and mean body mass index 22.0 ± 1.9 kg/m^2; [47]). In this study, rosmarinic acid was absorbed, conjugated, and methylated following intake and a small proportion of rosmarinic acid was degraded into various constituents, including conjugated forms of caffeic acid, ferulic acid, and m-coumaric acid, which were rapidly excreted in urine.

Several studies have examined the effects of rosmarinic acid on allergic reactions in mice. In nasal mucosa of ovalbumin-sensitized BALB/c mice, rosmarinic acid and 30% ethanol extract powder of perilla reduced the number of ear and eye rubs, the levels of IgE and histamine in the serum, inhibited protein and mRNA expression of interleukins (IL)-1β, IL-6, and tumor necrosis factor-α, and inhibited cyclooxygenase-2 protein expression and caspase-1 activity [48]. Moreover, rosmarinic acid blunted nuclear factor-kappa B (NF-κB)/Rel A and caspase-1 activation. These results suggested that both rosmarinic acid and 30% ethanol extract powder of perilla alleviate allergic inflammatory reactions such as allergic rhinitis and allergic rhinoconjunctivitis. Consistent with this report, rosmarinic acid ameliorated type-I allergies in ddY mice [49] and inhibited ear-passive cutaneous anaphylaxis (PCA) reactions more effectively than the modern antiallergic drug tranilast.

In HIGA mice that spontaneously develop high levels of serum immunoglobulin A (IgA) and mesangial IgA deposition, rosmarinic acid suppressed serum IgA levels and may suppress IgA nephropathy [18].

In forced swimming tests, rosmarinic acid produced an antidepressant-like effect, at least in part via the proliferation of new-born cells in the dentate gyrus of the hippocampus [50]. Rosmarinic acid also reduced the defensive freezing behaviour of mice exposed to conditioned fear stress [51]. The authors suggest that investigating the mechanisms of these effects may help to explain the pathophysiology of such affective disorders, which would facilitate new therapeutic strategies and future development of novel anxiolytic and/or antidepressive drugs.

In other studies, rosmarinic acid demonstrated anticarcinogenic effects in a murine two-stage skin model [52]. This paper concluded that rosmarinic acid contributes to the anticarcinogenic effects of perilla via two independent mechanisms: by inhibiting inflammatory responses and potent superoxide scavenging activity. Futhermore, rosmarinic acid ameliorated LPS-induced liver injury in D-GalN-sensitized mice [53].

In vitro, rosmarinic acid inhibited mesangial-cell proliferation [54] and adriamycin-induced apoptosis in H9c2 cardiac muscle cells [55]. Moreover, Kim et al. suggested that rosmarinic acid should be viewed as a potential chemotherapeutic that inhibits cardiotoxicity in adriamycin-exposed patients. Indeed, rosmarinic acid significantly prevented 6-OHDA-induced cell viability reduction [56], indicating potential as a chemotherapeutic agent for the treatment of Parkinson’s disease. Finally, rosmarinic acid inhibited the formation of reactive oxygen and nitrogen species in RAW264.7 macrophages [44].

2.6. Other constituents

Perillaketone has been shown to activate cloned TRPA1 channels *in vitro* [37]. Bassoli et al. [37] suggested that these data may explain the taste properties of the plant at the molecular level and identify it as an interesting target for its culinary and pharmaceutical applications.

Carvane was shown to inhibit the transformation of *Candida albicans* [36] and also inhibited type-I allergies in ddY mice [18].

Luteolin inhibits inflammation and allergic responses *in vivo* and *in vitro* [57] and the mechanisms of relaxant action were elucidated in isolated guinea pig tracheas [58].

The perilla constituent 1,2-Di-O-alpha-linolenoyl-sn-glycerol, which was extracted from the local variety, kida-chirimen shiso, inhibited superoxide generation[59].

2.7. Discussion

In this review, we were not able to find investigations on dietary perilla, though numerous studies revealed bioactive constituents of perilla.

We searched literature from medicine, pharmacology, and nutritional sciences to clarify the functions of perilla. We found very few studies of perilla on humans but many *in vivo* and *in vitro* investigations of its biological properties. Whereas dietary effects of perilla remain unknown, perilla oil is considered a high quality oil in nutritional sciences, which are concerned with lifestyle-related diseases.

According to numerous *in vivo* and *in vitro* studies, perilla and its constituents have anticancer, antiallergy and antidepressant properties, and the biological activities of perilla and its constituents are increasingly well characterised (Table 3). Although there are several *in vitro* studies demonstrating anticancer properties, studies conducted *in vivo* are very few. Hence, further research is required to examine the effects of using perilla as a food and medicine in daily life.

Among active constituents of perilla, perillaldehyde and rosmarinic acid have received the most attention, suggesting that these compounds are important constituents. Perillaldehyde contributes to aroma and accounts for approximately 50% of the oil refined from perillas. Therefore, it is considered important to examine the effects of this compound on stress; as for Kampo and food, its pleasant aroma is well known. However, only one paper considers bioactivity from aroma alone [31]. As rosmarinic acid is a constituent of both perilla and rosemary, scientific data is available for rosmarinic acid than for the other perilla compounds. Consequently, its effects on allergies are well characterised in humans [46].

In this review, we have summarized the current progress in perilla research. Although we found abundant *in vitro* and animal studies, we could not find a human study regarding the traditional theory on the effects of Kampo aroma on stress and depression. Investigating and clarifying the physiological effects of perilla and its constituents on humans are imperative in the future to adhere to the ideals of evidence-based medicine (EBM).

2.8. Conclusion

Animal and *in vitro* studies abundantly demonstrate the effects of perilla and its constituents; however, their influence on humans is nearly unknown. In contrast, perilla has already been used as a form of oriental medicine and as food. Investigating and clarifying the physiological effects of perilla and its constituents on humans are imperative in the future to adhere to the ideals of EBM.

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2011;75:2240–2242.

Table 1. Types of a perilla used for food and medicinal purposes (Kitagawa et al. 2011, Makino 2008)

	Scientific name
Food	*Perilla frutescens* (L.) Britton *crispa* (Thunb.) H. Deane f. *purpurea* (Makino) Makino
	Perilla frutescens (L.) Britton *crispa* (Thunb.) ‘*Viridi-crispa*’
	Perilla frutescens (L.) *frutescens*
Medicine Perillae Herba	*Perilla frutescens* Britton var. *acuta* Kudo
	Perilla frutescens Britton *crispa* var. Decaisne

Table 2. The main constituents in Perillae Herba (Kitagawa et al. 2011)

	Compounds
Essential oil	(−)-perillaldehyde, (+)-perillalcohol, (+)- limonene, alpha-pinene, and trans-shisool
Pigment	Cyanin and shisonin
Other compounds	Rosmarinic acid, adenine, and arginine

Table 3. The main effects of perilla

Product studied	Main effects	Type of study	Number of papers
Perilla decoction	Type I antiallergic effect Mouse ear passive cutaneous anaphylaxis (PCA) reaction IgA nephropathy in HIGA mice Decreased proliferation of mesangial cells	animal	5
Perilla extract	Inhibits growth and induces NO production in vascular smooth muscle cells	*in vitro*	2
Perilla oil	Protective effects against recurrent aphthous stomatitis (−)	human	1
	Fatty acid synthesis, Improved learning in the Sidman active avoidance task	animal	8
	Cytostatic activity	*in vitro*	1
Perillaldehyde	Antidepressant-like effect through regulation of the olfactory nervous system	animal	2
	Vasodilatory effect Cytostatic activity and so on	*in vitro*	6
Perillyl alcohol	Cytostatic activity	*in vitro*	2
Perillic acid	Cytostatic activity	*in vitro*	2
Rosmarinic acid	Seasonal allergic rhinoconjunctivitis inhibition, absorption, metabolism, degradation and urinary excretion	human	2
	Antiallergic effects against type-I allergies Antidepressant-like effect Anticarcinogenic effect	animal	8
	Cytostatic activity	*in vitro*	4
Caffeic acid	Absorption, metabolism, degradation, and urinary excretion	human	1
	Antidepressant-like effect	animal	1

3. Olfactory stimulation

3.1. Introduction to olfactory stimulation

The result of our research involving perilla indicated that there was no evaluation data on human physiological comfort for olfactory stimulations. Therefore, data accumulation for olfactory stimulations is essential. Therefore, we performed an experiment to clarify the physiological effects of olfactory stimulations using perilla essential oils.

Next, we focused on rose and orange essential oils, used throughout the ages, and performed olfactory-stimulation experiments. One of the most often used fresh flowers throughout Japan is the chrysanthemum; however, we chose the rose, which is the second most popular flower after the chrysanthemum, because the chrysanthemum is known as the Buddha flower in Japan. We found very little physiological data on either rose or orange essential oils in olfactory stimulations. Physiological effects of natural environments and products have attracted much interest in recent years. However, modern civilization makes regular contact with natural environments difficult. Thus, the relaxation effects of natural products that can be used on a daily basis are of increasing interest. People have been aware of essential oils, which are derived from plants, for a long time. Recently, we have become interested in the effects of daily exposure to essential oils as a natural therapy. Aromatherapy, the practice of using essential oils in a therapeutic context, is increasingly common, and rose and orange oils are particularly popular. Several studies have investigated the effects of these essential oils. Previous studies of the effects induced by rose and orange oils have used several physiological and/or subjective indices [1-8]. However, there has been no report of prefrontal cortex activity measured every second with near-infrared time-resolved spectroscopy (TRS). TRS measurement is able to detect the absolute value of brain activity; thus, it differs from other brain activity measurements [9-11]. The aim of the present study was to assess the effects of olfactory stimulation with rose or orange oil on left and right prefrontal cortex activity using near-infrared spectroscopy (NIRS) and also to determine the subjective effects of inhalation of the odors of these oils.

On the other hand, essential oils are easy to use for olfactory stimulations. The process of extracting essential oils adds heat by steam distillation, presenting volatilization at low boiling points. Therefore, we performed olfactory-stimulation experiments using actual fresh rose flowers to resolve this problem. In modern societies, individuals are exposed to many stressors in daily life [12-15]; thus, many individuals seek contact with nature to relax. One mode of achieving contact with nature is viewing fresh flowers such as roses. Roses are one of the most popular flowers, are a common gift for women, and are used to decorate rooms. The effects of olfactory stimulation with rose extracts, including rose essential oil, have been investigated using both physiological and subjective evaluations [6, 8-10, 16, 17]. The present study is novel in several aspects. First, no previous study has been performed using fresh rose flowers. Second, no other study has evaluated the physiological effects of olfactory stimulation by fresh rose flowers on women from the perspective of autonomic nervous activity, particularly by assessing heart rate variability (HRV), which is a measure of sympathetic and parasympathetic nervous activity. Finally, we performed a qualitative analysis of the characteristic odor of *Rosa hybrida* “Meikarouz,” also known as “Rouge Royale” [18]. The aim of the present study was to characterize the effect of olfactory stimulation by fresh rose flowers, which exude a strong fragrance, on HRV.

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3.2. Effects of olfactory stimulation with perilla essential oil on prefrontal cortex activity

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ABSTRACT

Objectives: The objective of the present study was to clarify the effect of olfactory stimulation with perilla essential oil on the human prefrontal cortex activity using near-infrared time-resolved spectroscopy.

Design: This was a single-center prospective study.

Setting, Subjects, Intervention, and Outcome Measures: Nineteen female university students (21.6 ± 1.5 years old) participated in the study. We used perilla essential oil as an olfactory stimulant and air as a control. Oxyhemoglobin concentrations in the prefrontal cortex were measured using near-infrared time-resolved spectroscopy when the subjects were presented with the stimulant or control. Subjective evaluations of emotional impact were evaluated using a modified semantic differential method.

Results: The oxyhemoglobin concentration in the prefrontal cortex was significantly reduced by olfactory stimulation with the perilla essential oil. No significant differences in the feelings of comfort or relaxation, which are strongly reflective of human mental status, were observed between the perilla essential oil and control.

Conclusions: Our results clearly indicate that olfactory stimulation with the perilla essential oil reduced the prefrontal cortex activity, thereby inducing physiological relaxation.

Introduction

Perilla has been used as a traditional medicine and functional food item since ancient times [1, 2]. In traditional medicine, aromatic substances are often used to treat mental stress [3]. Perilla is one such substance that is also used in combination with other aromatic traditional medicines referred to as Kampo medicines (hereafter “Kampo”).

Hange-koboku-to (Chinese name: Banxia-houpo-tang), Koso-san (Chinese name: Xiang-Su-San), and Saiboku-to are representative of Kampo and are used to treat depression-related diseases [1, 4]. In previous animal studies, Koso-san exhibited antidepressant-like effects [5], and Saiboku-to potentiated the inhibition of cerebral acetylcholine release induced by diazepam [6]. However, little is known on the effects of these Kampo in humans and on the role of perilla. Most Kampo have been used as medicines for a long time and have been approved for use as general medicines, including perilla, which does not have any status as a medical drug with proven efficacy, but has been approved as an herbal medicine.

Traditional medicine is of increasing interest as an alternative medicine worldwide, and the World Health Organization has announced that it will add a chapter on traditional medicines, including the Japanese Kampo, to the 11th edition of the International Classification of Diseases (ICD-11) in 2015. Accordingly, a number of scientific investigations on the effects of Kampo have been initiated. Nonetheless, the importance of perilla remains to be clarified. Perillaldehyde, which is the primary component of perilla, has been reported to have an antidepressant-like effect via the olfactory nervous system *in vivo* in a murine model [7]. However, till date, the physiological effects of olfactory stimulation with perilla in humans have not yet been identified [8].Given the tendency toward an increase in the number of individuals suffering from stress-related diseases worldwide [9, 10],determining the physiological effects of perilla and its ability to induce relaxation is important.

We had determined previously the physiological effects of nature on human physiology and quality of life [11-16] and that near-infrared time-resolved spectroscopy is a useful technique for performing such studies [17-19]. Measurement of the prefrontal cortex activity is possible at 1 Hz because the change in oxyhemoglobin (oxy-Hb) concentration can be measured using this method.

Accordingly, the aim of the present study was to determine the effects of olfactory stimulation with perilla on the human prefrontal cortex activity.

Materials and Methods

Subjects

In this experiment, 19 female university students (age range, 21.6 ± 1.5 years) participated. All of them were informed about the aim and procedures involved in the experiment, and they provided their written informed consent to participate. This study was performed in accordance with the regulations of the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan.

Study protocol

Physiological measurements were performed in a chamber with an artificial climate maintained at 25°C with 50% relative humidity and 230 lux illumination. We used perilla essential oil (Tree of Life Co., Ltd.; extracted from the leaves and subjected to steam distillation) as an olfactory stimulant and air as a control. Perilla essential oil (0.5 μL) was injected in a 24-L odor bag; odors were presented to each subject by means of a device fixed on the chest and situated approximately 10 cm under the nose (Fig. 1). The flow rate of the essential oil was set at 3.0 L/min. Preliminary investigations determined the subjective intensity to odor, for example, weak or easily sensed. Our investigations determined that odor intensities should be neither too weak nor too strong. The odor was administered for 90 s while the subjects sat with their eyes closed. The order of presentation of the odor and control was counterbalanced for each subject.

Near-infrared time-resolved spectroscopy

During the odor administration, oxy-Hb concentrations in the prefrontal cortex were measured using the near-infrared time-resolved spectroscopy (TRS) via the TRS-20 system (Hamamatsu Photonics K.K.) [17-19]. The oxy-Hb concentrations in the left and right prefrontal cortex were measured at 1 Hz for 10 s before (pre-measurement condition) odor administration as well as during the 90 s of odor administration (post-measurement condition). The oxy-Hb concentration shown is the difference between the pre- and post-measurement conditions. Data were transformed by linear interpolation because the 1 Hz sampling rate was only an approximate. We used the difference in oxy-Hb concentrations between the pre- and post-measurement conditions for analysis.

Semantic differential method

In addition to the neurophysiological measurements, the subjects provided a subjective evaluation of the emotional impact of the odors using a modified semantic differential (SD) method [20]. The modified SD method used three pairs of adjectives assessed on 13 scales, including “comfortable–uncomfortable,” “relaxed–awakening,” and “natural–artificial.” The SD rating test was performed after odor administration.

Statistical analysis

The oxy-Hb concentration in the post-measurement condition was compared with that in the pre-measurement (average 10 s) condition to determine any variation, and assessments were made every 30 s. All experiments were set at a 1-s interval from 0 to 1 s.

Statistical Package for Social Sciences software (v21.0, IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Neurophysiological responses to the perilla essential oil and control were compared with a two-way analysis of variance (ANOVA) for repeated measures followed by analysis of simple main effects. Wilcoxon signed-rank test was applied to analyze differences in psychological indices between the perilla essential oil and control.

Results

Physiological effects

We measured oxy-Hb concentrations in the prefrontal cortex of women during olfactory stimulation with the perilla essential oil (Fig. 2). We used the two-way ANOVA with oxy-Hb concentrations as the dependent variable, and olfactory stimulation and elapsed time as the independent variables. The two-way ANOVA revealed no significant effect of the olfactory stimulus (perilla essential oil vs. control) or the administration time on oxy-Hb concentrations, but a significant olfactory stimulus × time-interaction effect in the left prefrontal cortex [$F(2,28) = 4.842$; $p < 0.05$]. Similarly, there was no significant effect of the olfactory stimulus or time, but a significant stimulus × time-interaction effect in the right prefrontal cortex [$F(2,28) = 9.993$; $p < 0.01$]. Because the stimulus × time-interaction effect was significantly different in both the left and right prefrontal cortex, we assessed the simple main effects using a post-hoc test assessing every 30 s of elapsed time.

No significant simple main effect regarding the oxy-Hb concentration in the left prefrontal cortex was observed between 1 s and 30 s and between 31 s and 60 s. However, between 61 s and 90 s, the oxy-Hb concentration was −0.25 μM during the administration of the perilla essential oil and 0.14 μM during the administration of the control; analysis of the 0.39 μM difference indicated that the oxy-Hb concentration was significantly lower following exposure to the perilla essential oil compared with the control [F (1,14) = 5.345; $p < 0.05$] (Fig. 2). Similarly, there was no significant simple main effect regarding the oxy-Hb concentration in the right prefrontal cortex between 1 s and 30 s and 31 s and 60 s. However, between 61 s and 90 s, the oxy-Hb concentration was −0.57 μM after exposure to the perilla essential oil and −0.11 μM after exposure to the control {0.46 μM was significantly different; [F (1,14) = 5.193; $p < 0.05$]} (Fig. 2). The above findings clearly indicate that olfactory stimulation with the perilla essential oil reduced activity in the prefrontal cortex.

Psychological effects

Subjective reports of feeling “comfortable,” “relaxed,” and “natural” were determined using the modified SD method (Fig. 3). There was no difference in the reports of feelings of comfort or relaxation between the perilla essential oil and control, all of which were reported to lie between “indifferent” and “slightly comfortable” or “slightly relaxed” on the measurement scale. However, reports of feeling “natural” ranged from “indifferent” to “slightly natural” with the perilla essential oil but from “indifferent” to “slightly artificial” with the control. The perilla essential oil was therefore perceived as being significantly more natural than the control.

Discussion

Most reports concerning perilla have been associated with both *in vivo* and *in vitro* studies. Perillaldehyde, which is the bioactive component of perilla, has been shown to have an antidepressant-like effect via the olfactory nervous system in an *in vivo* mouse model.[7] Perillaldehyde is assumed to be an important component associated with the a priori stress relief experienced when exposed to odors because it is a specific odorous substance that constitutes approximately 50% of the composition of the perilla essential oil. Thus, it has been suggested that perilla odor is bioactive *in vivo*. Furthermore, studies targeting humans are required for its daily use.

In this study, we determined the effect of olfactory stimulation with the perilla essential oil on the human prefrontal cortex activity. We found that olfactory stimulation with the perilla essential oil reduced the oxy-Hb concentrations and decreased the right and left prefrontal cortex activity (oxy-Hb concentration was used as an index of activity). This suggests that perilla can make a significant contribution toward improving the effects of stress in an increasingly artificial society and that it can be incorporated into everyday use.

With regard to the subjective evaluations, feelings of comfort and relaxation are strongly reflective of the human mental state. In this study, the subjects reported feeling “indifferent” or “slightly comfortable” or “slightly relaxed” when exposed to both the perilla essential oil and control; whereas, the physiological indices showed a clear decrease in both the left and right prefrontal cortex activity. Thus, the physiological evaluation was superior to subjective evaluation.

We have previously studied the effects of nature on the human physiology and have confirmed that activity in the prefrontal cortex is decreased in forest therapy studies [21, 22]. We have also reported a decrease in sympathetic nervous activity, an increase in parasympathetic nervous activity, and decreases in blood pressure and heart rate because of exposure to nature [12, 13]. Furthermore, we have reported decreased concentrations of cortisol, a typical stress hormone, and improved immune functioning in individuals with a weakened immune system as a result of forest therapy [23-25]. Thus, it is clear that natural forested environments have physiological effects on humans, including induction of physiological feelings of relaxation. In this study, we only evaluated the prefrontal cortex activity; therefore, this cannot be considered a complete physiological evaluation. Other experimental indices, such as brain activity, autonomic nervous activity, endocrine activity, and immune activity, should now be assessed to determine the effects of olfactory stimulation with perilla on human physiology and feelings of relaxation.

Conclusion

Compared with the control, olfactory stimulation with perilla essential oil was found to significantly reduce the oxy-Hb concentrations and the prefrontal cortex activity, but no significant difference in the feelings of comfort or relaxation were reported in the subjective evaluations. In conclusion, it is clear that olfactory stimulation with the perilla essential oil reduced the prefrontal cortex activity, thereby inducing physiological relaxation.

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Fig. 1. The scene during olfactory stimulation, and the device used to administer the odors

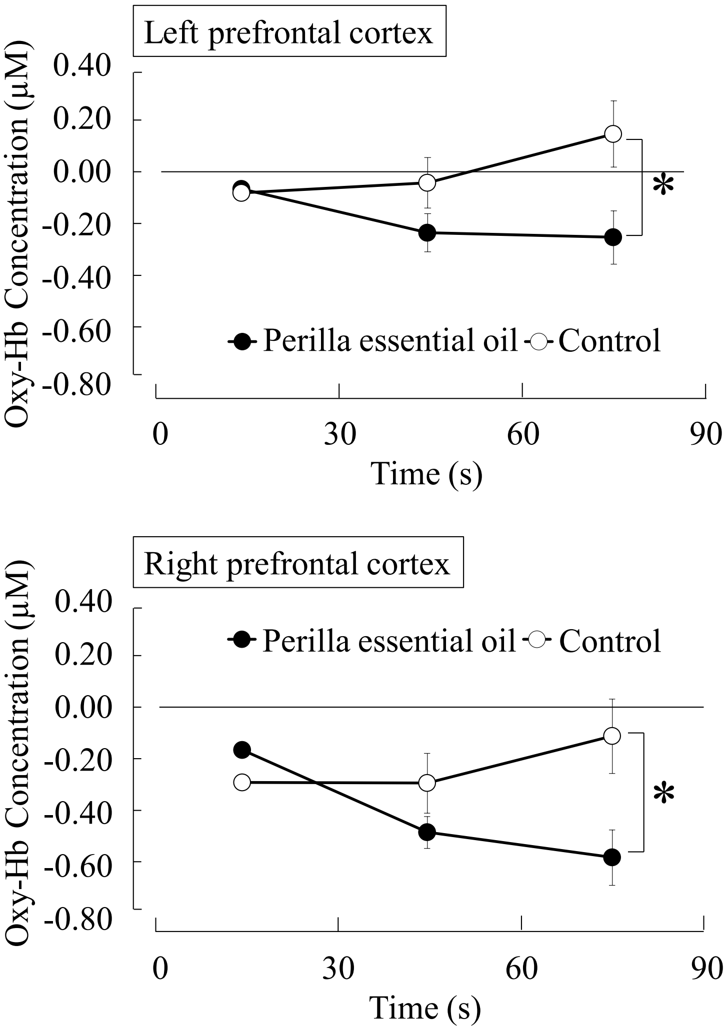

Fig. 2. Time-dependent oxyhemoglobin (oxy-Hb) concentration changes in the prefrontal cortex during olfactory stimulation with the perilla essential oil or control (air). The oxy-Hb concentration shown is the difference between the pre- and post-measurement conditions. Data are expressed as mean ± standard error (SE); n = 15. * $p < 0.05$ by the two-way analysis of variance (ANOVA) for repeated measures, simple main effects.

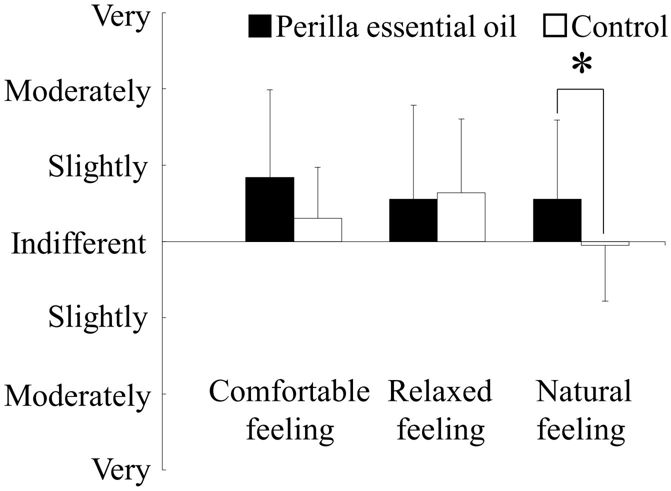

Fig.3. Subjective feelings measured by a modified semantic differential (SD) questionnaire after olfactory stimulation with perilla essential oil or the control. Data are expressed as mean ± standard deviation (SD); n = 19. *$p < 0.05$ by Wilcoxon signed rank test

3.3. Effects of olfactory stimulation with rose and orange oil on prefrontal cortex activity

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Abstract

Objectives: People have been aware of essential oils, which are derived from plants, for a long time. Recently, we have become interested in physiological and subjective effects of daily exposure to essential oils. The primary aim of the present study was to clarify effects of olfactory stimulation with rose or orange oil on prefrontal cortex activity; subjective evaluations of relaxation were also determined.

Setting and Interventions: Subjects were exposed for 90 s to air impregnated with either rose or orange essential oil. As a control, subjects wore the same device but inhaled only unimpregnated air. The three stimuli were randomly presented to each subject.

Main outcome measures: Physiological effects were determined by near-infrared time-resolved spectroscopy and a modified semantic differential approach was used to determine subjective evaluations.

Results: The study participants were 20 female university students (mean age 22.5 ± 1.6 years). Olfactory stimulation by rose or orange oil induced: (1) a significant decrease in oxyhemoglobin concentration in the right prefrontal cortex and (2) an increase in “comfortable,” “relaxed,” and “natural” feelings.

Conclusion: These findings indicate that olfactory stimulation by rose or orange oil induces physiological and psychological relaxation.

Introduction

Physiological effects of natural environments and products have been of much interest in recent years. Forest therapy (walking in or viewing forests) has, for example, been demonstrated to induce relaxation effects in a number of field experiments [1-5].

However, modern civilization makes regular contact with natural environments difficult. Accordingly, the relaxation effects of natural products that can be used on a daily basis are of increasing interest.

People have been aware of essential oils, which are derived from plants, for a long time. Recently, we became interested in the effects of daily exposure to essential oils as a natural therapy. Aromatherapy, which is the practice of using essential oils in a therapeutic context, is increasingly common and rose and orange oils are especially popular. Several studies have investigated the effects of these essential oils.

There have been relatively few studies of the physiological effects of rose odor on humans. Inhalation of the odor of rose oil was shown to decrease relative sympathetic activity as measured by heart rate variability and adrenaline concentration in healthy normal adult females [6]. In addition, Fukui *et al.* [7] reported that rose odor inhalation decreased salivary cortisol levels in healthy college students. Moreover, Fukada *et al.* [8] showed that inhaling the odor of rose essential oil inhibited increases in salivary cortisol and skin-barrier disruption in healthy female college students exposed to stress (university examinations). Regarding subjective evaluations of effects, Ayan *et al.* [9] showed that inhalation of the odor of rose essential oil is a useful supplementary and adjunctive therapy for relieving renal colic in renal colic patients.

In humans, the physiological effect of olfactory stimulation with the odor of orange essential oil, which is a subjectively pleasant odor, on P3 of the event-related potential (ERP), which concerns mental activity, is different from that of eugenol, which is a subjectively unpleasant odor [10]. Moreover, orange essential oil has been shown to reduce anxiety and improve mood in a dental office [11]. In addition, healthy female school-age children have been reported to be more likely to feel happy when smelling sweet orange essential oil [12, 13].

Previous studies of the effects induced by rose and orange oils have used several physiological and/or subjective indices. However, there has been no report of prefrontal cortex activity measured every second with near-infrared time-resolved spectroscopy (TRS). The TRS measurement is able to detect the absolute value of brain activity, and thus differs from other brain activity measurements [14-16].

The aim of the present study was to clarify the effects of olfactory stimulation with rose or orange oil on left and right prefrontal cortex activity using near-infrared spectroscopy (NIRS) and also to determine the subjective effects of inhalation of the odors of these oils.

Methods

All study participants provided their written informed consent for participation after they were informed about the study's aims and procedures. The study was performed in accordance with the regulations of the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan. Physiological measurements were performed in a chamber with an artificial climate maintained at 25°C with 50% relative humidity and 230 Lux illumination. In the artificial climate chamber, air is exchanged approximately every minute through ventilation. In this study, air was exchanged through ventilation approximately every 7 min and between treatments for each subject.We used rose essential oil (Tree of Life Co., Ltd.; *Rosa damascena*, product of Bulgaria, extracted from the flowers and subjected to solvent extraction) or orange essential oil (Tree of Life Co., Ltd.; *Citrus sinensis*, product of Brazil, extracted from the peels and compressed) as an olfactory stimulant and air as a control. Air impregnated with rose (0.2 μL) or orange (0.7 μL) essential oil was injected into a 24-L odor bag (polyethylene terephthalate film heat seal bag; NS-KOKEN Co., Ltd. Kyoto, Japan) and presented to each subject with a device that rested on the subject's chest approximately 10 cm under the nose (Fig. 1). The flow rate of the air impregnated with essential oil was set at 3.0 L/min. Preliminary investigations were used to determine subjective intensity to odor, for example, slight or weak sensation. Our investigations determined that odor intensities should be neither too weak nor too strong. The odor was administered for 90 s while the subjects sat with their eyes closed. The order of presentation of the three stimuli was random for each subject.

Physiological effects were determined by measuring oxy-hemoglobin (oxy-Hb) concentrations in the prefrontal cortex using near-infrared TRS (TRS-20 system, Hamamatsu Photonics K.K.; [14-16]). The oxy-Hb concentrations in the left and right prefrontal cortex were measured at 1 Hz for 10 s before (pre-measurement condition) odor administration as well as during the 90 s of odor administration (post-measurement condition). Post-measurement values (every second) were compared with the pre-measurement value (mean 10 s) and differences determined. Differences were calculated based on absolute oxy-Hb concentration values and were not extrapolated since TRS enables detection of absolute values. Furthermore, we calculated a mean value per 90 s using differences in oxy-Hb concentrations. Data were transformed by linear interpolation because the 1 Hz sampling rate was only approximate.

In addition to the neurophysiological measurements, subjective evaluations of the emotional impact of the odors were determined using a modified semantic differential (SD) method [17]. Three pairs of adjectives were assessed on 13 scales, including "comfortable–uncomfortable," "relaxed–awakening," and "natural–artificial." The SD rating test was performed after odor administration.

Statistical Package for Social Sciences software (V20.0; IBM Corp., Armonk, NY, USA) was used for all statistical analyses. A paired t-test with a Holm correction was used to compare physiological responses to the rose oil, orange oil, and control. Wilcoxon's signed-rank test with a Holm correction was applied to analyze differences in the psychological indices of responses to the rose oil, orange oil, and control. In both cases, one-sided tests were used because of the hypothesis that humans would be relaxed by the odor of rose or orange oil. The Holm correction was applied twice (i.e. a Holm correction was applied between the control and the rose oil and between the control and the orange oil).

Results

The study participants were 20 female university students (mean age, 22.5 ± 1.6 years). Time-dependent oxy-Hb concentration changes per 1 s in the prefrontal cortex during olfactory stimulation by rose essential oil, orange essential oil, and the control are shown in Figure 2. The comparisons of the mean oxy-Hb concentrations in the prefrontal cortex per 90 s during olfactory stimulation by rose essential oil, orange essential oil, or the control are shown in Figure 3. At 90s, the mean oxy-Hb concentration in the right prefrontal cortex was −0.14 μM after exposure to the control, −0.55 μM after exposure to rose oil (a 0.41-μM significant decrease ; $p < 0.05$) and −0.76 μM after exposure to orange oil (a 0.62-μM significant decrease; $p < 0.05$; Fig.3). Olfactory stimulation by rose or orange oil induced a significant decrease in oxy-Hb concentrations in the right prefrontal cortex compared with the control. In the left prefrontal cortex, although there was a tendency toward a decrease in oxy-Hb concentrations, there was no significant difference.

A modified SD method was used in this study to provide subjective reports of “comfortable,” “relaxed,” and “natural” feelings (Fig. 4). These subjective reports indicated that the subjects felt “slightly” to “moderately” comfortable, relaxed, and natural when exposed to the odors of rose or orange oil but “indifferent” or “slightly” comfortable, relaxed, and natural when exposed to the control. Moreover, olfactory stimulation by both stimuli indicated that inhalation of rose or orange oil was perceived as significantly comfortable, relaxed, and natural ($p < 0.05$, Fig. 4).

Discussion

Olfactory stimulation by rose or orange oil induced a significant decrease in oxy-Hb concentrations in the right prefrontal cortex compared with the control. In the left prefrontal cortex, although there was a tendency toward a decrease in oxy-Hb concentrations, there was no significant difference. Homae [18] highlighted the functional organization of the two hemispheres by reviewing some of the most recent functional NIRS studies that have reported hemispheric differences in activation patterns. Most NIRS studies using visual stimuli that have revealed functional differences between the hemispheres have reported unilateral activation, that is, significant levels of activation in one hemisphere only. Auditory stimuli, including speech sounds, elicit bilateral activation, while a limited number of studies of young infants have revealed primarily unilateral activation in these subjects. Much regarding NIRS measurement of the effects of olfactory stimuli remains unknown and requires further study.

In this study, we determined the effects of olfactory stimulation with rose or orange oil on human prefrontal cortex activity. We found that olfactory stimulation with rose or orange oil reduced oxy-Hb concentrations and decreased activity in the right prefrontal cortex (oxy-Hb concentration was used as an index of activity). We have previously elucidated the effects of natural stimulants on human prefrontal cortex activities in field studies that involved activities such as staying in a forest for a period of time [19]. Exposure to natural environments has also been reported to increase parasympathetic nervous activity [2, 20-25], decrease sympathetic nervous activity [2, 21, 22, 24, 25], and decrease concentrations of cortisol, which is a typical stress hormone [19, 21-26]. These findings strongly suggest that exposure to nature can induce physiological relaxation.

In the present study, we notified subjects before they participated in the experiment that they would receive nature-derived olfactory stimulation. Thus, the experiment was not performed under blind conditions. However, future studies similar to the present study should be performed under blinded conditions.

In an artificial climate chamber, air is exchanged approximately every minute through ventilation. In this study, air was exchanged through ventilation approximately every 7 min and between treatments for each subject. With this ventilation rate, we could examine each odor presentation without the influence of the prior presentation.

Previous studies that examined rose essential oil as an olfactory stimulant used heart rate variability, adrenaline concentration [6], salivary cortisol levels [7, 8], and subjective evaluation [9] as measurement indices. These measures clarified the effects of rose essential, and they suggest that rose essential oil may have beneficial effects. With regard to orange essential oil, there are few reported physiological effects [10], whereas most of the reported data include subjective evaluation [11, 12, 13].

Nevertheless, orange essential oil may have beneficial effects. In this study, we found that rose and orange essential oils could decrease prefrontal cortex activity. In light of these physiological effects, essential oil may be used more frequently in the future in daily life and medical settings to help induce physiological relaxation.

In this study, we only evaluated prefrontal cortex activity and this cannot be considered a complete physiological evaluation. Other experimental indices, such as autonomic nervous activity and stress hormone levels, should now be assessed to more comprehensively determine the effects of olfactory stimulation with rose or orange oil on human physiology.

In conclusion, olfactory stimulation by rose or orange oil induced: (1) a significant decrease in oxy-Hb concentrations in the right prefrontal cortex and (2) an increase in subjective reports of “comfortable,” “relaxed,” and “natural” feelings. These findings indicate that olfactory stimulation by rose or orange oil induces physiological and psychological relaxation.

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Figures


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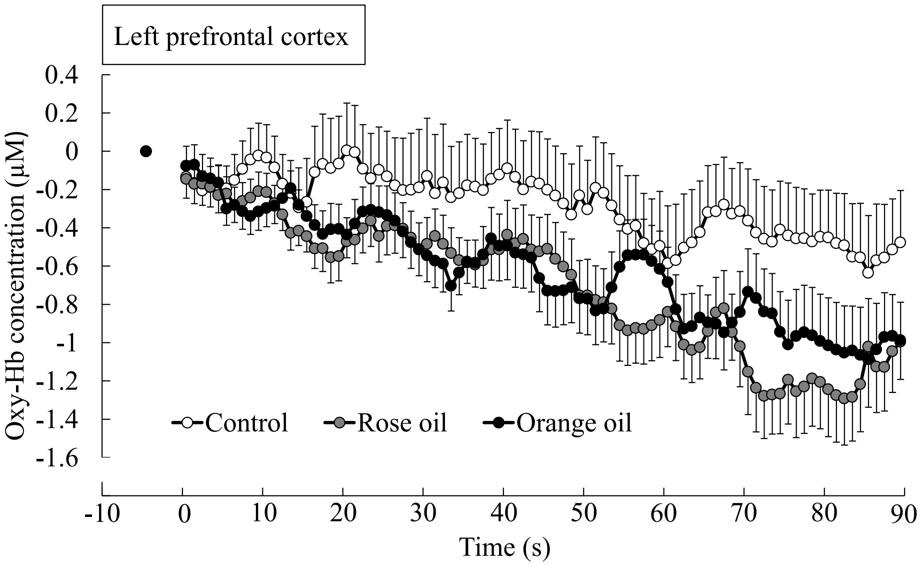
Fig.1 The scene during olfactory stimulation and the device used to administer the odors.



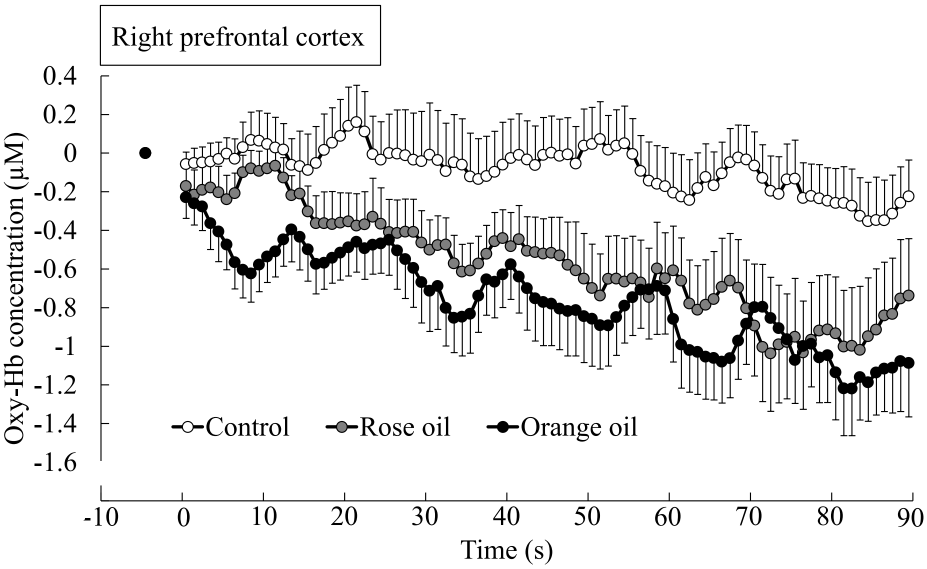


Fig.2. Time-dependent oxy-Hb concentration changes per 1 s in the prefrontal cortex during olfactory stimulation by rose essential oil, orange essential oil, or the control. The oxy-Hb concentration shown is the difference between the pre- and post-measurement conditions. Data are expressed as the mean ± SE; n = 15.

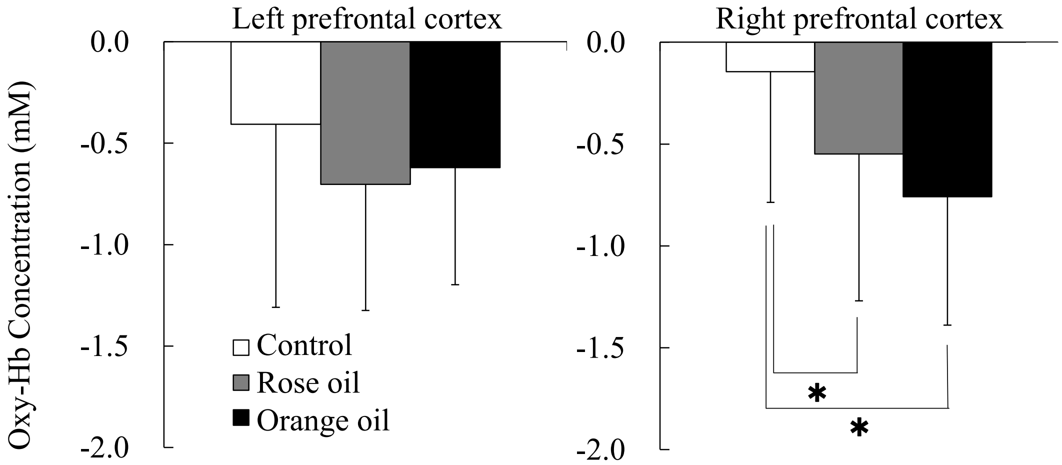


Fig.3. Comparison of mean oxy-Hb concentrations in the prefrontal cortex over 90 s during olfactory stimulation by rose essential oil, orange essential oil, or the control. The oxy-Hb concentration shown is the difference between the pre- and post-measurement conditions. Data are expressed as the mean ± SD; n = 15. *:$p < 0.05$ by t-test (one-side) with a Holm correction. The Holm correction was performed twice (i.e. the Holm correction was applied between the control and the rose oil and the control and the orange oil).

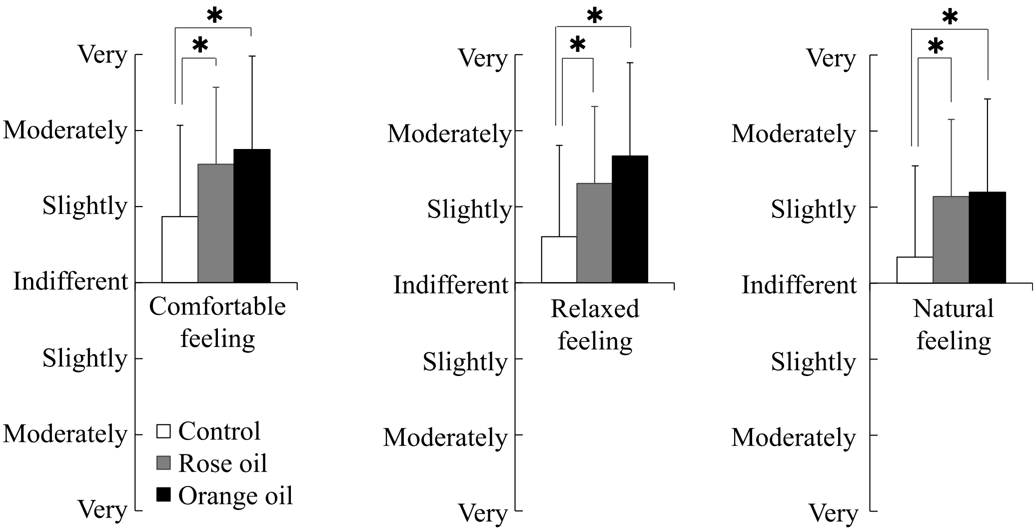


Fig. 4. Subjective feeling as measured by a modified semantic differential (SD) questionnaire after olfactory stimulation by rose oil, orange oil, or the control. Data are expressed as the mean ± SD; n = 18. *:$p < 0.05$ by Wilcoxon signed-rank test (one-side) with a Holm correction. The Holm correction was performed twice (i.e. the Holm correction was applied between the control and the rose oil and the control and the orange oil).

3.4. Effect of olfactory stimulation with fresh rose flowers on autonomic nervous activity

J Altern Complement Med. 2014 Sep;20(9):727-31.
doi: 10.1089/acm.2014.0029.

Abstract

Objective: The aim of the present study was to clarify the effect of olfactory stimulation by fresh rose flowers, which exude a strong fragrance, on heart rate variability.

Settings/Location: A chamber with an artificial climate maintained at 25°C with 50% relative humidity and 230 lux illumination at the Center for Environment, Health, and Field Sciences, Chiba University, Japan.

Subjects: Nineteen female university and graduate students (mean age: 21.6 ± 1.5 years, range: 19.0–26.0 years).

Interventions: Fresh rose flowers as an olfactory stimulant, with air as a control.

Outcome Measures: Heart rate variability and subjective evaluations. The power levels of the high frequency (HF) (0.15–0.40 Hz) and low frequency (LF) (0.04–0.15 Hz) components of heart rate variability were calculated by the maximum-entropy method. The HF power was considered to reflect parasympathetic nervous activity. The LF/HF power ratio was determined to reflect the sympathetic nervous activity. A modified semantic differential method was used to perform subjective evaluations.

Results: Fresh rose flowers induced: (1) a significant increase in parasympathetic nervous activities and (2) an increase in “comfortable” and “natural” feelings.

Conclusion: The findings indicated that olfactory stimulation by fresh rose flowers induced physiological and psychological relaxation.

Introduction

In modern societies, individuals are exposed to many stressors in daily life [1-4], and consequently, many individuals seek contact with nature to relax. One means of achieving the contact with nature is viewing fresh flowers, such as roses, which is one of the most popular flowers and is a common gift for women and is used to decorate rooms.

We have previously shown that visual stimulation by fresh rose flowers (*Rosa* 'Dekora') induces physiological relaxation in female medical staff [5]. To date, there have been a few studies that have evaluated the physiological effects of the odor of rose on humans. Inhalation of the odor of rose essential oil (*Rosa damascena* MILL.) decreases relative sympathetic activity and adrenaline concentration in normal adults [6]. Moreover, Stankewitz *et al*. explored neuronal processing in response to olfactory stimulation with rose oil, in patients with migraine using event-related functional magnetic resonance imaging (fMRI) and found that odor of rose oil induces significantly higher blood oxygen level-dependent signal intensities in several brain areas during spontaneous and untreated migraine attacks [7]. In addition, Fukui *et al.* reported that rose odor (essential oil of *Rosa alba*) inhalation decreases cortisol levels in healthy volunteers [8]. Moreover, Fukada *et al.* showed that inhaling odor of rose essential oil (*Rosa alba*) inhibits increases in salivary cortisol and skin-barrier disruption in humans exposed to stress [9]. Regarding subjective evaluations of effects, Ayan *et al.* used a visual analog scale of pain scores ranging from "no pain" to "very severe pain" to demonstrate that inhalation of odor of rose essential oil (*Rosa damascena*) is a useful supplementary and adjunctive therapy for relieving renal colic [10]. Thus, the effects of olfactory stimulation by rose extracts, including rose essential oil, have been investigated using both physiological and subjective evaluations.

The present study is novel with regard to a number of aspects. First, no previous study has been performed using fresh rose flowers. Second, no other study has evaluated the physiological effects of olfactory stimulation by fresh rose flowers on women from the perspective of autonomic nervous activity, particularly by assessing heart rate variability (HRV), which is a measure of sympathetic and parasympathetic nervous activity. Finally, we conducted a qualitative analysis of the characteristic odor of *Rosa hybrida* “Meikarouz,” also known as “Rouge Royale [11].”

As mentioned above, HRV has been used as a measure of autonomic nervous activity. The maximum-entropy method (MemCalc/Win; GMS, Tokyo, Japan) was used to calculate the power levels of high frequency (HF, 0.15–0.40 Hz) and low frequency components (LF, 0.04–0.15 Hz). The HF power was considered to reflect parasympathetic nervous activity, and the LF/HF power ratio was considered to reflect sympathetic nervous activity [12, 13]. Physiological relaxation effects were evaluated using various previously described stimuli [14-19].

The aim of the present study was to clarify the effect of olfactory stimulation by fresh rose flowers, which exude a strong fragrance, on HRV.

Material and Method

Subjects

In this experiment, 19 healthy female volunteers were recruited from approximately 500 students from our university and graduate school (mean age: 21.6 ± 1.5 years, range: 19.0–26.0 years). We excluded subjects with breathing disorders, such as colds and nasal inflammation. Subjects were requested to get sufficient sleep the day before the experiment, to avoid drinking alcohol, and to control their conditions. All were informed of the aims and procedures involved in the experiment and provided written informed consent to participate. This study was performed in accordance with the regulations of the Ethics Committee of the Center for Environment, Health, and Field

Sciences, Chiba University, Japan.

Study protocol

Physiological measurements were performed in a chamber with an artificial climate maintained at 25°C with 50% relative humidity and 230 lux illumination [20]. We used fresh rose flowers (*Rosa hybrida* "Meikarouz," also known as "Rouge Royale,"[11] from Enomoto Rose Garden, Japan) as an olfactory stimulant and air as a control. Four flowers were put into a 24-L odor bag (polyethylene terephthalate film heat seal bag; NS-KOKEN Co., Ltd. Kyoto, Japan) and the odors were presented to each subject by means of a device fixed on the chest and situated approximately 10 cm under the nose (Fig. 1). The flow rate of the odor was set at 3.0 L/min. Preliminary investigations determined the subjective sensitivity to odor, for example, weak or easily sensed. Our investigations determined that odor intensities should be neither too weak nor too strong. We examined different variations of roses and selected four. The odor was administered for 90 s while the subjects sat with their eyes closed. We performed a within-subject experiment. To eliminate the effect of the order of olfactory stimulation, approximately half of participants were administrated stimuli in the following order: control, then rose. The remaining participants were then presented with the rose first, then control.

Heart rate variability

As a physiological measurement, HRV was analyzed for the periods between consecutive R waves in the electrocardiogram (RR intervals) as measured by a portable electrocardiograph (Activtracer AC-301; GMS, Japan) [21, 22]. This device performs measurements using measures 3-leadelectrocardiogram (lead II). The power levels of the HF (0.15–0.4 Hz) and the LF (0.04–0.15 Hz) components of HRV were calculated by the maximum-entropy method (MemCalc/Win; GMS, Japan). The HF power was considered to reflect parasympathetic nervous activity. Furthermore, the LF/HF power ratio was determined to reflect the sympathetic nervous activity. We analyzed the means of data acquired for 90 s.

Semantic differential method

Regarding psychological measurements, to provide a subjective evaluation of the emotional impact of the odors, subjects were tested by the modified semantic differential (SD) method [23]. The modified SD method used two pairs of adjectives assessed on 13 scales, including "comfortable–uncomfortable," and "natural–artificial."

Measurement of volatile organic compounds

Volatile organic compounds emitted from the flowers were collected in PEJ-02 tubes (Supelco Inc.) by enclosing one weighed flowers inside a 5.0-L Tedlar bag (GL Science Inc.) [24]. The air flow rate through the bag was approximately 80 mL/min, and the temperature of the bag was 25.0 ± 2.0°C. The collected volatile compounds were removed from the PEJ-02 tube by heating the trap with an Automatic Thermal Desorption System (ATD400, Perkin Elmer) at 280°C for 15 min. The compounds were cryofocused in a cold trap (air monitoring trap) at 2°C. By heating the cold trap, volatiles were transferred to a HP-5MS capillary column (30 m × 0.25 mm i.d.× 0.25-μm film thickness, Agilent Technology) and analyzed using gas chromatography-mass spectrometry (GC-MS, Hewlett Packard GC type 6890, MSD 5973) [25]. The temperature program for GC-MS was 40°C for 15 min, from 40°C to

180°C at 4°C/min, 180°C for 15 min, from 180°C to 280°C at 5°C/min, and 280°C for 15 min. All mass numbers between 15 and 550 m/z were recorded (SCAN technique). Individual compounds were identified by comparing their mass spectra with the NIST Library. Literature values and NMR spectra were detailed as in a previous report [26].

Statistical analysis

SPSS software, version, 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The HRV power level data were presented as means ± SE. A paired t-test was used to compare physiological responses to the fresh rose flowers and controls. Wilcoxon's signed-rank test was applied to analyze differences in psychological indices between the responses to the fresh rose flowers and to the control. In both cases, we applied one-sided tests because of the hypothesis that humans would be relaxed by fresh rose flowers.

Results

Physiological effects

Figure 2A shows the HF value associated with olfactory stimulation by fresh rose flowers. When the results of the HRV power level data were compared, a significant difference was found in the HF power level between the fresh rose flowers and the control ($p = 0.0495$). The HF power level of fresh rose flowers (527.36 ± 99.02 msec^2) was 19.2% higher than that of the control (442.54 ± 97.11 msec^2). It was clear that the olfactory stimulation by the fresh rose flowers induced a significant increase in parasympathetic nervous activities, thereby inducing physiological relaxation. Furthermore, the LF/HF power ratio of the fresh rose flowers (1.00 ± 0.25) was 43.8%

lower than that of the control (1.78 ± 0.59). The difference was not significant but the results suggested that fresh rose flowers induced a trend towards decreased sympathetic nervous activities ($p = 0.0595$, Fig. 2B).

Psychological effects

The modified SD method was used to provide subjective reports of “comfortable” and “natural” feelings (Fig. 3). The subjective reports of feeling “comfortable” and “natural” “very comfortable” and “slightly natural” for fresh rose flowers but as “slightly comfortable,” and “indifferent” for the control. The response to fresh rose flowers was therefore perceived as being significantly more comfortable and natural than that to the control ($p < 0.05$).

Volatile compounds

The volatile compounds from the fresh rose flowers are shown in Table 1. The predominant compounds in the fresh rose flowers were limonene, 2-phenethyl acetate, and citral. The physiological effects of these compounds remain unknown and further detailed studies are required.

Discussion

In this study, we measured the effects of olfactory stimulation by fresh rose flowers in humans. Our results showed that this induced physiological and psychological effects. There were some studies on the effects of olfactory stimulation by rose essential oils [6-10]. Also, there were many studies on the rose variety Rosa damascene [6, 10], but there were no previous studies on the physiological effect and component analysis of

“Rouge Royale,” which is a relatively new rose created in the 2000s [11]; therefore, this study is novel with regard to this aspect.

Similar to this report, we have previously studied the effects of nature on human physiology [5, 14-18, 27, 28]. We have also reported a decrease in sympathetic nervous activity [13, 14, 16-18], an increase in parasympathetic nervous activity [5, 14-19], a decrease in prefrontal cortex activity [28], and decreased concentrations of stress hormones in forest therapy studies [14-17, 19, 27, 28]. In the future, these indices should be comprehensively assessed to determine the effects of olfactory stimulation by fresh rose flowers on human physiological effects and feelings of relaxation. In the current study, we chose ‘Rouge Royal’, which has a strong fragrance. In the future, it will be necessary to clarify the different effects of other rose varieties. A previous report showed that the main odor components differ between varieties [29]. Therefore, it is expected that physiological effects by every component should be clarified.

The main limitation of the study is that we evaluated sympathetic and parasympathetic nervous activity by HRV only as indices of physiological relaxation. Other experimental indices, such as prefrontal cortex activity and stress hormone concentrations, should also be assessed for a more comprehensive determination of the effect of olfactory stimulation by fresh rose flowers on human physiology.

In conclusion, our results showed that olfactory stimulation by fresh rose flowers induced (1) a significant increase in parasympathetic nervous activities that are enhanced a relaxed state, (2) a trend toward decreased sympathetic nervous activities, and (3) an increase in “comfortable” and “natural” feelings.

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Figures

Fig. 1. The scene during olfactory stimulation and the device used to administer the odors.

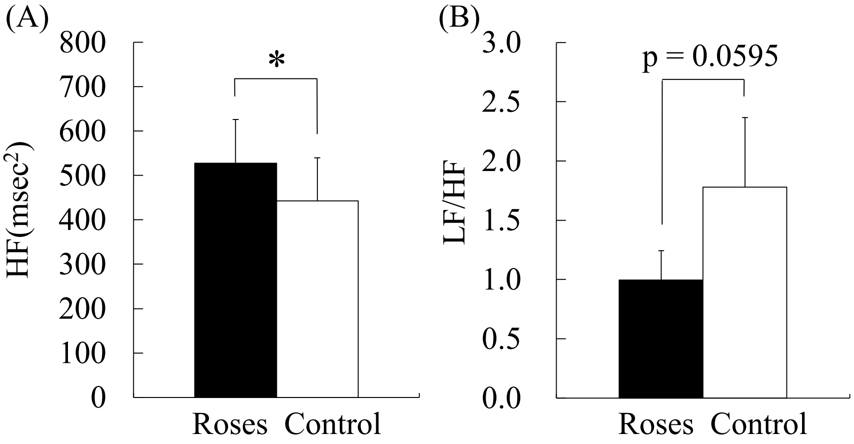

Fig.2. Comparison of high-frequency power levels and low-frequency/high-frequency power level ratios of heart rate variability during olfactory stimulation by fresh rose flowers or control. Data are expressed as means ± SE; n = 16. * $p < 0.05$ by paired t-test (one-sided).

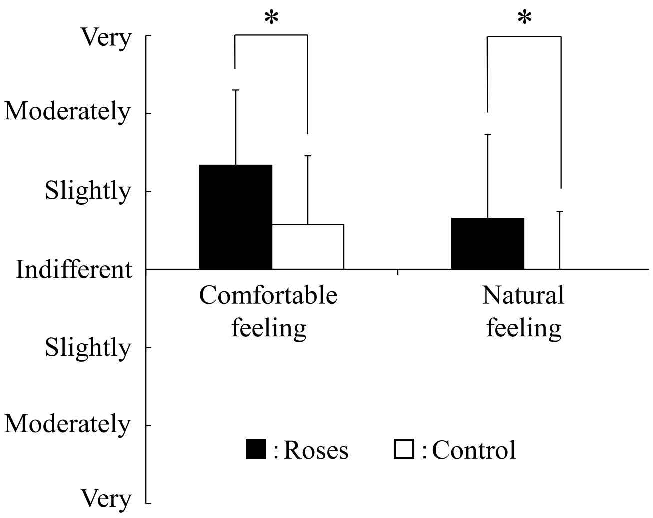

Fig. 3. Subjective feeling measured by the modified semantic differential questionnaire after olfactory stimulation by fresh rose flowers or control. Data are expressed as means ± SD; n = 19. * $p < 0.05$ by Wilcoxon signed-rank test (one-sided).

Table 1. Volatile compounds emitted from the fresh rose flower

Identified compounds	Relative area (%)
Ethanol	4.0
Methoxy-benzene	3.6
acetic acid	2.5
α-Pinene	1.6
Camphene	1.4
β-Pinene	1.3
Methoxymethyl benzene	3.1
β-Terpinene	4.8
β-Myrcene	5.8
Hexyl acetate	4.6
o-Ocimene	7.0
Limonene	13.8
β-Ocimene	4.0
α-Ocimene	7.2
Terpinolene	1.8
δ-2-Carene	1.8
4-α-Dimethylstyrene	4.5
Cyclofenchene	1.6
3,4-Dimethyl-2,4,6-octatriene	1.9
β-Citronellal	2.7
2-Phenethyl acetate	9.1
Citral	9.0
δ-Cadinene	1.2

3.5. Conclusion of olfactory stimulation

3.2. Effects of olfactory stimulation with perilla essential oil on prefrontal cortex activity

Olfactory stimulation with perilla essential oil significantly reduced prefrontal cortex activity, thereby inducing physiological relaxation.

3.3. Effects of olfactory stimulation with rose and orange oil on prefrontal cortex activity

Olfactory stimulation by rose or orange oil significantly reduced right prefrontal cortex activity, thereby inducing physiological relaxation.

3.4. Effect of olfactory stimulation with fresh rose flowers on autonomic nervous activity

Olfactory stimulation by fresh rose flowers induced a significant increase in parasympathetic nervous activities, thereby inducing physiological relaxation.

To clarify the physiological responses of plant-derived olfactory stimulations, we studied the effects of using perilla, rose, and orange essential oils, and fresh rose flowers. Our results confirmed that olfactory stimulations with perilla, rose, and orange essential oils calmed the brain’s prefrontal cortex activities, while the olfactory stimulation using fresh rose flowers increased parasympathetic nerve activity. However, the finding that rose and orange essential oil decreased brain activity in the right but not in the left hemisphere was very interesting. Considerable information about the brain activity of olfactory stimuli remains unknown and requires further study. The plant-derived olfactory stimulations clearly had physiological relaxation effects.

3. Visual stimulation

4.1. Introduction to Visual Stimulation

This chapter focuses on plant-derived visual stimulations, with specific focus on the physiological effects. Imaging techniques provide an opportunity to develop a realistic image, with familiar and convenient display images reflecting a plant of interest. Accumulating evidence indicates that photographic images of the natural environment (in contrast to the urban built environment) can have a beneficial effect on stress physiology and cognitive restoration. In addition to the lack of opportunity and contemporary restrictions (real or perceived) on the amount of time available for nature-based recreation, there are other situations where visually provided elements of nature may provide a convenient alternative to a natural environment.

No comparative studies have used large two-dimensional (2D) display images and actual plant physiological data. Recent developments in image projection technology have enabled us to visualize images such as natural environments with greater clarity. Thus, familiar and convenient visual stimulation derived from nature is possible. However, it remains unknown whether the physiological effects of stimulation by foliage plant images are comparable to those by real foliage plants. Hence, we studied the differences between actual foliage plants and display image stimulations using familiar foliage plants commonly that are used in offices and homes.

While 3D images have recently attracted attention due to their high interpretations as real, there were no physiological relaxation effects from 3D plant image stimulations. To date, most researchers have investigated either 2D photographic images alone or, in separate studies, 3D scenes as a part of virtual reality therapeutics. Nonetheless, there have been little or no data for comparison of 2D and 3D elements of nature because they relate to physiological endpoints. Because 3D imagery may provide a greater sense of reality and immersion in a natural environment (or fascination with a singular element of nature such as attractive flora), we hypothesize that more pronounced changes in stress physiology are associated with 3D than with 2D images. The findings of this study not only support evolutionary “appreciation” of specific flora but also suggest that 3D nature imagery is helpful when walking in a forest is not a viable option. The aim of this study was to compare physiological responses to various 3D visual image stimulations of plants and then compare those responses with 2D images.

Previous questionnaire studies revealed that 3D images increased ratings for motion sickness such as nausea and disorientation compared with 2D images [1, 2]. In contrast, changes in the low frequency (LF) component of HRV relative to the high frequency component (HF; LF/HF ratio), a measure of sympathetic nerve activity, did not differ significantly between 3D and 2D images [1]. To the best of our knowledge, no study has evaluated the physiological relaxation effects of 3D flower images based on prefrontal cortex and autonomic nerve activity. Thus, the present study aimed to clarify the effects of visual stimulation by 3D images on left and right prefrontal cortex activity assessed using near-infrared spectroscopy (NIRS) [3-5] and autonomic nerve activity assessed using HRV [6, 7] and determine the subjective effects of 3D images compared with those of 2D images.

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4.2. Effect of stimulation by foliage plant display images on prefrontal cortex activity: A comparison with stimulation using actual foliage plants

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ABSTRACT

Natural scenes like forests and flowers evoke neurophysiological responses that can suppress anxiety and relieve stress. We examined whether images of natural objects can elicit neural responses similar to those evoked by real objects by comparing the activation of the prefrontal cortex during presentation of real foliage plants with a projected image of the same foliage plants. Oxy-hemoglobin concentrations in the prefrontal cortex were measured using near-infrared time-resolved spectroscopy while the subjects viewed the real plants or a projected image of the same plants. Compared with a projected image of foliage plants, viewing the actual foliage plants significantly increased oxy-hemoglobin concentrations in the prefrontal cortex. However, using the modified semantic differential method, subjective emotional response ratings (“comfortable vs. uncomfortable” and “relaxed vs. awakening”) were similar for both stimuli.The frontal cortex responded differently to presentation of actual plants compared with images of these plants even when the subjective emotional response was similar. These results may help explain the physical and mental health benefits of urban, domestic, and workplace foliage.

Introduction

Human evolution is an adaptation to nature; therefore, natural scenes have significant effects on brain activity and physiological responses, including stress responses [1]. However, because of progression in society over the past few centuries, humans have experience stressful situations due to urbanization and artificial environment [2, 3, 4, 5]. To date, we have accumulated large amounts of data on the physiological relaxation effect derived from various aspects of nature [6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. In contrast, recent developments in image projection technology have enabled us to visualize images such as natural environments with greater clarity. Accordingly, familiar and convenient visual stimulation derived from nature is expected. However, it remains unknown whether the physiological effects of stimulation due to foliage plant images are comparable to those due to real foliage plants. We examined potential differences in neurophysiological responses to natural and projected scenes by measuring activation of the prefrontal cortex in response to presentation of foliage plants and projected images of the same plants.

Materials and Methods

Eighteen female university students (21.6 ± 1.5 years old) participated in this experiment. They were sufficiently informed about the aim and the procedure of this experiment, and all subjects provided their written informed consent. This study was performed according to the regulations of the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan. Physiological measurements were performed in an artificial climate chamber maintained at 25 ºC with 50% relative humidity and 300 lux illumination. For foliage plants, we used three dracaena plants (*Dracaena deremensis*). Subjects viewed the three plants, sitting side-by-side on the floor or a high-resolution image of the three plants projected at the same size, luminosity, and position on a TV screen (58V type, TH-58PZ800 by Panasonic). Fig. 1 shows the study protocol, Fig. 2 the scene at rest (control, a box), and Fig. 3 the scene during visual stimulation (the plants). The subjects viewed the stimuli in the following order: an actual cardboard box for 30 s (Fig. 2, left), actual dracaena plants for 3 min (Fig. 3, left), a projected image of the cardboard box for 30 s (Fig. 2, right), and finally, the projected image of the dracaena plants for 3 min (Fig. 3, right). The images were then presented in a different order (Fig. 1, lower). While viewing the plants and images, oxy-hemoglobin (oxy-Hb) concentrations in the prefrontal cortex were measured using near-infrared time resolved spectroscopy (TRS) using the TRS-20 system (Hamamatsu Photonics K.K.) [16, 17]. The oxy-Hb concentrations in the left and right prefrontal cortex were measured at 1 Hz for the last 10 s of box presentation (real or image, termed the pre-measurement condition) and during the entire 3-min presentation of the plant or plant image (post-measurement condition). Data were transformed by linear interpolation because the 1 Hz sampling rate was only approximate. We used the oxy-Hb concentration difference between the pre- and post-measurements for analysis. We excluded approximately 30 s of data acquired while the assessor removed the box from the experimental scene, because it would cause cortical activation that was unrelated to object perception. In addition to the neurophysiological measurements, subjects gave a subjective evaluation of the emotional impact of the image and plant using the modified semantic differential (SD) method [18]. The SD method uses three pairs of adjectives on 13 scales, including "comfortable–uncomfortable," "relaxed–awakening," and "natural–artificial." This SD rating test was performed after presentation of the visual stimuli (Fig. 1).

Statistical Package for Social Sciences software (v21.0, IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Neurophysiological responses to the plants and images were compared by two-way analysis of variance (ANOVA) for repeated measures, followed by analysis of simple main effects and multiple comparisons with the Holm correction. The Wilcoxon signed-rank test was applied to analyze differences in psychological indices between the two visual stimuli (plants or image).

Results and Discussion

We measured oxy-Hb concentrations in the prefrontal cortex of healthy women while they viewed real dracaena plants or a projected image of the same plants (Fig. 4). Two-way ANOVA revealed a significant effect of the visual stimulus (real vs. image) [F (1,15) = 6.292, $p < 0.05$], a significant effect of viewing time [F (2,30) = 15.805, $p < 0.01$], and a significant stimulus × time interaction in the left prefrontal cortex [F (2,30) = 6.424, $p < 0.01$). Similarly, there was a significant effect of visual stimulus [F (1,15) = 6.299, $p < 0.05$], time [F (2,30) = 11.913, $p < 0.01$] , and stimulus × time interaction [F (2,30) = 6.945, $p < 0.01$] in the right prefrontal cortex. In the left prefrontal cortex, simple main effects analysis revealed that the average oxy-Hb concentrations were significantly higher while viewing the actual dracaena plants compared with the projected image over the 1–2 min interval [F (1,15) = 7.919, $p < 0.05$] and over the 2−3 min interval [F (1,15) = 7.269, $p < 0.05$] . Moreover, multiple comparison tests indicated significantly higher mean oxy-Hb concentrations during the 1−2 min and the 2−3 min intervals compared with the 0−1 min interval for the real dracaena plants ($p < 0.01$ for both intervals), but not for the projected image. The oxy-Hb concentration changes were similar in the right prefrontal cortex, with significantly higher concentrations in response to the real dracaena plants compared with the image [1−2 min interval: F (1,15) = 6.047, $p < 0.05$; 2−3 min interval: F (1,15) = 6.884, $p < 0.05$] and compared with the 0−1 min interval while viewing the actual plants (1−2 min: $p < 0.01$; 2−3 min: $p < 0.05$). Despite the distinct cortical responses, the subjective feelings elicited by the two stimuli were similar (Fig. 5), with both the real plants and image inducing slightly “comfortable” and “ relaxed” feelings. The actual dracaena plants were rated as significantly more “natural,” as expected ($p < 0.01$).

We report the first demonstration of a differential cortical response to actual foliage plants compared with an image of the same plants. Viewing actual foliage plants significantly increased oxy-Hb concentrations in the prefrontal cortex, whereas the projected image did not. Surprisingly, both the real plants and the image induced feelings of comfort and relaxation as measured by the modified SD method. Given the role of the prefrontal cortex in emotional regulation, the actual plants may have psychological benefits not replicated by the image. These results underscore the possible benefits of plants for stress relief in homes and offices. Future studies will examine if this prefrontal activation is dependent on plant volume, number, or species. Perhaps certain plants are more “anxiolytic” than others.

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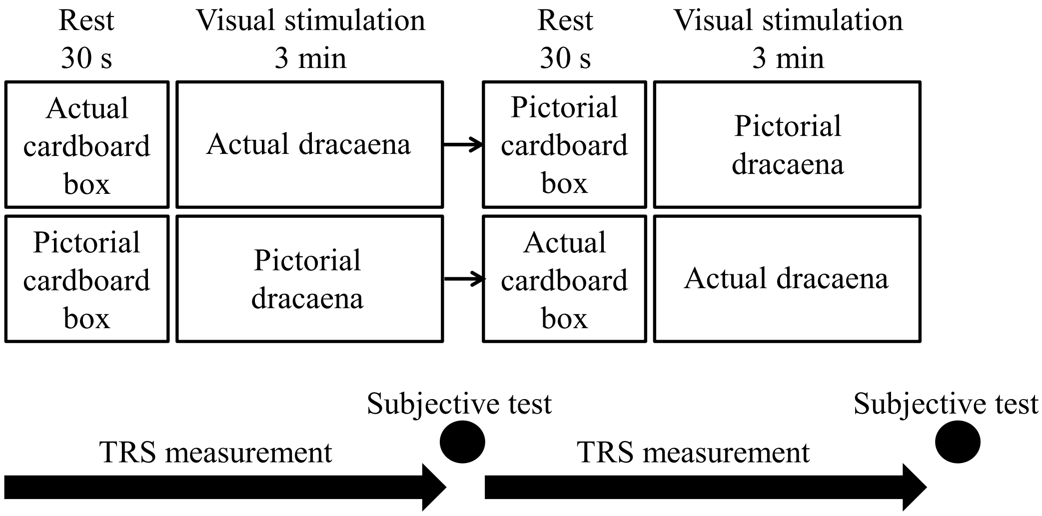


Fig. 1. Study protocol.

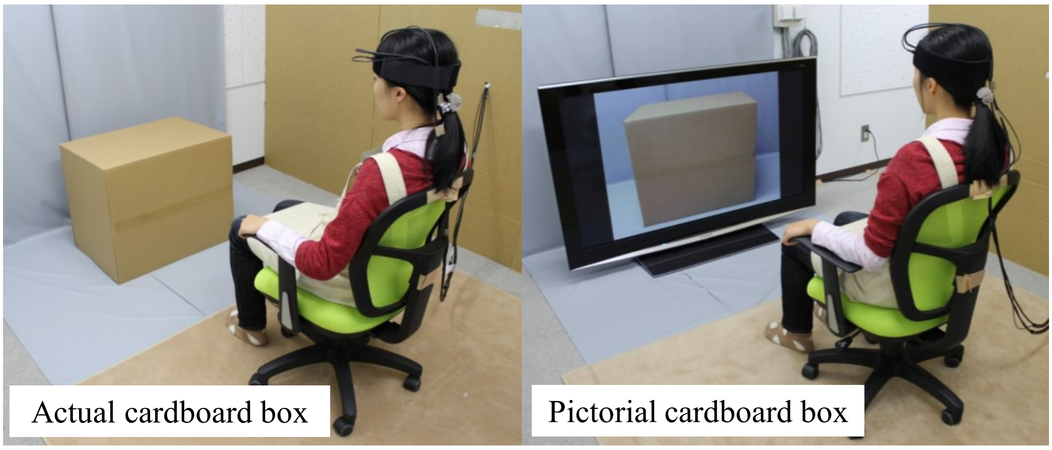
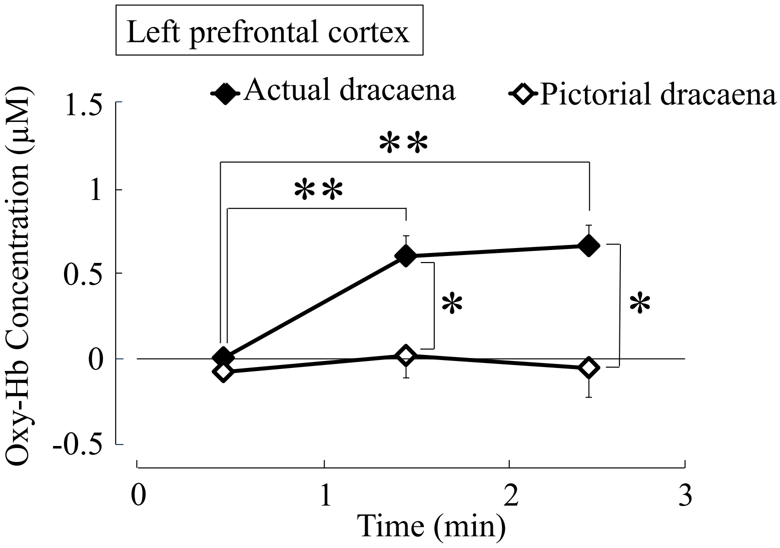


Fig. 2. The scene at rest.

Fig. 3. The scene during visual stimulation.



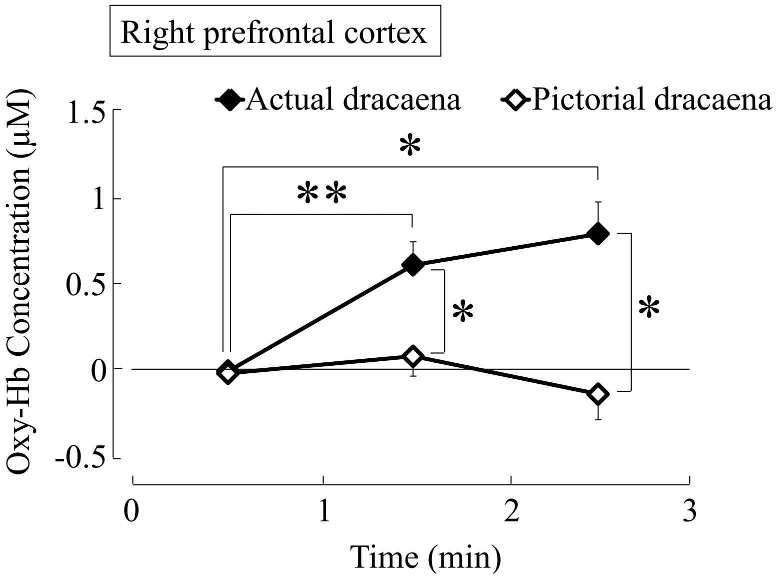


Fig. 4. Time-dependent oxy-Hb concentration changes in the prefrontal cortex while viewing dracaena plants or a projected image of the plants. The oxy-Hb concentration shown is the difference between the pre- and post-measurement conditions. Data are expressed as mean ± SE; n = 16. *$p < 0.05$, **$p < 0.01$ by two-way analysis of variance (ANOVA) for repeated measures with post-hoc Holm correction.

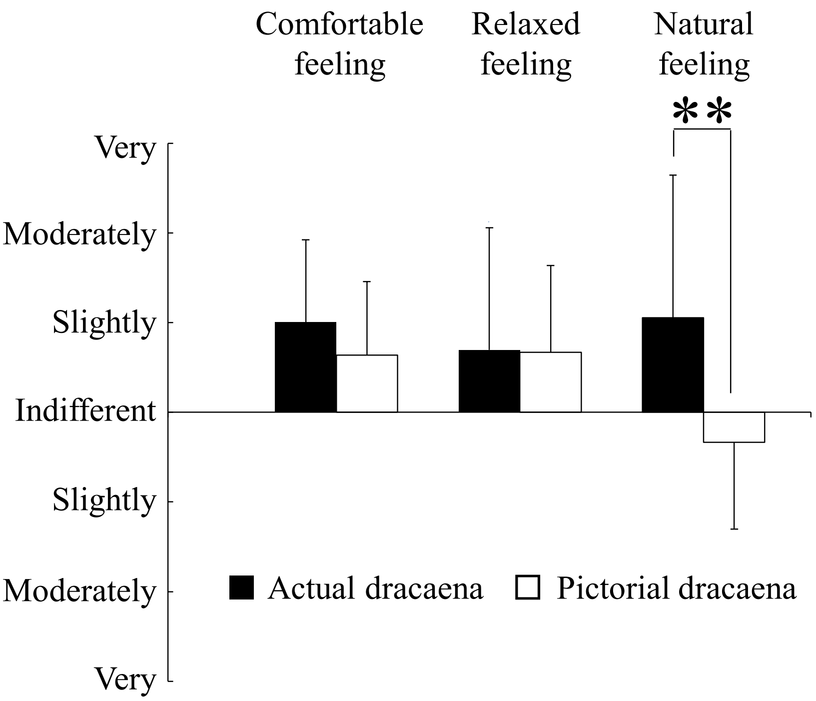


Fig. 5. Subjective feeling measured by the modified semantic differential (SD) questionnaire after viewing real dracaena plants or an image of the plants. Data are expressed as mean ± SD; n = 18. **p < 0.01 by Wilcoxon signed-rank test.

4.3. Effects of stimulation by three-dimensional natural images on prefrontal cortex and autonomic nerve activity: a comparison with stimulation using two- dimensional images

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Abstract

Empirical evidence suggests that three-dimensional (3D) images of nature promote physiological relaxation in humans by providing more realistic effects compared with two-dimensional (2D) images. However, no studies have evaluated the physiological relaxation effects of nature-derived 3D images on prefrontal cortex and autonomic nerve activity. The present study aimed to clarify the physiological relaxation effects of visual stimulation by 3D flower images on prefrontal cortex and autonomic nerve activity. Nineteen male university students (22.2 ± 0.6 years) were presented with 3D and 2D images of the water lily for 90 s. Prefrontal cortex activity was measured using near-infrared spectroscopy (NIRS), while autonomic nerve activity was measured using heart rate variability (HRV). Psychological effects were determined using a modified semantic differential (SD) method. Compared with visual stimulation by 2D images, that by 3D images resulted in a significant decrease in oxyhemoglobin concentration in the right prefrontal cortex, lower sympathetic activity as calculated by the ratio of the low frequency (LF) to high frequency (HF) HRV component, and a significantly greater realistic feeling as evidenced by higher SD ratings. In conclusion, visual stimulation by realistic 3D floral images promotes physiological relaxation more effectively than the corresponding 2D image.

Introduction

Throughout the period of human evolution, spanning some 6 million years [1], our ancestors and we have been shaped by the natural environments in which we have sought shelter, safety, and sustenance. In the last century, and even more so in the last several decades, there has been rapid urbanization, such that the majority of humans are now living in built urban environments. Because 99.9% of our evolutionary past has involved an extremely close association with natural environments, it has been proposed that there may be health-related consequences of the mismatch between modern urban existence and the lack of opportunity/time for more direct contact with natural environments [2 modified]. In support of this hypothesis, several studies have shown that exposure to a natural environment such as a forested area ("forest therapy") promotes physiological relaxation, as measured by a variety of cardiovascular and neurophysiological [2-12]. We have demonstrated that time spent in a forest can decrease blood pressure [4, 10-12] and pulse rate [4, 5, 8, 10-12], suppress sympathetic nervous system activity [5, 8, 10-12], increase parasympathetic nervous system activity [4, 5, 8, 9, 11-13], decrease the salivary cortisol level (a stress hormone), and decrease cerebral blood flow in the prefrontal cortex [9].

Accumulating evidence indicates that photographic images of the natural environment (vs. urban built environment) can have a beneficial effect on stress physiology and cognitive restoration. In addition to the lack of opportunity and contemporary restrictions (real or perceived) on the amount of time available for nature-based recreation, there are a number of other situations where visually provided elements of nature may provide a convenient alternative to a natural environment. To date, most of the researchers have examined either 2-dimensional (2D) photographic images alone or, in separate studies, 3-dimensional (3D) scenes as a part of virtual reality therapeutics. Nonetheless, there has been little or no data in the way of comparison of 2D and 3D elements of nature as they relate to physiological endpoints. Because 3D imagery may provide a greater sense of reality and immersion in a natural environment (or fascination with a singular element of nature such as attractive flora), we hypothesize that there may be more pronounced changes in stress physiology associated with 3D images compared to 2D images. The findings of this study not only support evolutionary “appreciation” of specific flora but also indicate that 3D nature imagery may be helpful when walking in a forest is not a viable option.

Three dimensional (3D) images were first developed in the 19th century and were made extremely popular in the 1950s and 1980s. Furthermore, more realistic 3D images have been developed of late. Visualization of realistic 3D images of nature can evoke sensations similar to those elicited by actual nature in humans. Barfield et al. [14] mentioned that presence is generally defined as a users’ subjective sensation of “being there” in a depicted by a medium. Freeman et al. [15] indicated that users describe presence as a feeling of “being there” concerning 3D TV. Riva et al. [16] evaluated the link between presence and feeling, and showed that the level of presence influences the emotional state of the users. Nature scenes are often a part of existing therapeutic virtual reality, and some these experiments involved sound effects at the same time [17-19]. Gorini et al. [20] studied the use of a 3D image of the Green Valley, which is a natural environment, during a surgical operation. In the present study, we focused on the effects of images only (without auditory stimuli).

Previous questionnaire studies demonstrated that 3D images increased ratings for motion sickness such as nausea and disorientation compared with 2D images [21, 22].

In contrast, changes in the low-frequency (LF) component of heart rate variability (HRV) relative to the high-frequency component (HF; LF/HF ratio), a measure of sympathetic nerve activity, were not significantly different between 3D and 2D images [21].

To the best of our knowledge, no study has evaluated the physiological relaxation effects of 3D flower images on the basis of prefrontal cortex and autonomic nerve activity. Therefore, the present study aimed to clarify the effects of visual stimulation by 3D images on left and right prefrontal cortex activity assessed using near-infrared spectroscopy (NIRS) [23-25] and autonomic nerve activity assessed using heart rate variability (HRV) [26, 27]and determine the subjective effects of 3D images compared with those of 2D images.

Materials and Methods

Study protocol

We used the 3D dome scene simulation system at the National Institute for Rural Engineering, Japan. Images were presented using a liquid crystal display stereoscopic projection system consisting of two fisheye lenses (Tokyo Laboratory Ltd.; Patent number; 3452880). The projected images were 1.7-m long and 3.0-m wide, with a luminance of 10000 lm and average illumination of 2000 lx. The display was 2.5 m from the subject. Subjects first viewed a rural landscape picture, during which no physiological measures were acquired, followed by viewing 3D and 2D images of the water lily for 90 s. Figure 1 shows the visual stimuli and the viewing condition during stimulation. The order of 2D and 3D image presentation was counterbalanced for each subject.

Subjects

Nineteen male university students (22.2 ± 0.6 years old) participated in this experiment. They were informed of all experimental aims and procedures, and all subjects provided written informed consent. This study was performed according to regulations of the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan.

NIRS

We measured oxyhemoglobin (oxy-Hb) concentrations in the left and right prefrontal cortices using NIRS (NIRO-200, Hamamatsu Photonics K.K., Japan) [23-25]. The principles of the NIRS measurement are as follows. With the increase in local brain activity, brain blood flow increases, and luxury perfusion is produced such that the quantity of brain blood flow exceeds oxygen consumption [28]. Consequently, oxy-Hb increases, and this increase can be detected. In addition, it is known that increases or decreases in the quantity of brain blood flow are consistent with those of oxy-Hb [29], and it is thought that a decrease in the oxy-Hb concentration causes a physiological relaxation effect [30]. After confirming that the oxy-Hb concentration in the left and right prefrontal cortices became approximately constant for 10 s, we initiated visual stimulation. Oxy-Hb concentrations were measured at 1 Hz for 10 s before image presentation (prestimulus condition) and during the entire 90 s of visual stimulation (stimulus condition). The oxy-Hb concentration presented at each time point is the difference between the value at that time point and the mean pre-stimulus value.

HRV

HRV [26, 27] was continuously measures from the start to the end of image presentation by measuring the period between consecutive R waves (R−R intervals) using a portable electrocardiograph (Activtracer AC-301A; GMS, Japan). The power levels of the HF (0.15–0.40 Hz) and LF (0.04–0.15 Hz) components of HRV were calculated using the maximum entropy method (MemCalc/Win; GMS, Japan). The HF power is considered to reflect parasympathetic nervous activity, while the LF/HF power ratio is considered to reflect sympathetic nerve activity. In this study, we used the natural logarithmic value of the HF power or the LF/HF power ratio and analyzed the mean of values determined over the 90 s of image presentation.

Semantic differential method

In addition to physiological measurements, the participants subjectively evaluated the emotional impact of 3D and 2D images using a modified semantic differential (SD) method [31]. The SD method uses three pairs of adjectives on 13 scales: comfortable–uncomfortable, natural–artificial, and realistic–unrealistic. This SD rating test was conducted after presentation of each visual stimulus.

Statistical analysis

All statistical analyses were conducted using Statistical Package for Social Sciences V21.0 (IBM Corp., Armonk, NY, USA). The NIRS data are presented as means ± standard errors (SEs), while the HRV power level data are presented as means ± standard deviations (SDs). A paired t-test was used to compare physiological responses to 3D and 2D images. Wilcoxon signed-rank test was used to compare the psychological responses to the 3D and 2D images. In both cases, we applied one-sided tests because the predetermined hypothesis was that human indices of relaxation are more greatly affected by 3D flower image.

Results and Discussion

NIRS revealed that the metabolic response in the right prefrontal cortex while viewing a 3D image of the water lily was considerably different from that while viewing a similar 2D image (Fig. 2). The time-dependent oxy-Hb concentration changed little during the 90 s of viewing the 2D image, while it exhibited an obvious decrease during presentation of the 3D image. After 90 s of viewing, the mean oxy-Hb concentration in the right prefrontal cortex was 0.15 μM under the 2D image condition and −0.55 μM under the 3D image condition (a 0.70-μM decrease; $p < 0.010$; Fig. 3). There was no significant difference in oxy-Hb concentration in the left prefrontal cortex between the two viewing conditions (data not shown).

In addition to decreased metabolic activity in the right prefrontal cortex, the mean [ln(LF/HF)] value was lower while viewing the 3D image than while viewing the 2D image (0.23 vs. 0.59; $p < 0.05$; Fig. 4). In contrast, there was no significant difference in the HF value between the viewing conditions (data not shown).

The subjects rated the 3D image as “realistic,” “moderately realistic,” or “slightly realistic”, while they rated the 2D image as “indifferent” or “slightly unrealistic” (Fig. 5). Therefore, the 3D image was perceived to be significantly more realistic compared with the 2D image ($p < 0.01$; Fig. 5). However, there were no significant differences in subjective ratings for comfortable–uncomfortable and natural– artificial between the viewing conditions (data not shown).

In summary, visual stimulation by the 3D image induced a significant decrease oxyhemoglobin concentration in the right prefrontal cortex as assessed using NIRS, a significant decrease in [ln(LF/HF)], which reflects sympathetic nervous activity; and a significantly greater realistic feeling.

We previously reported physiological relaxation measured by a decrease in prefrontal cortex [9] and sympathetic nerve activities [3, 5, 10, 12, 13] during exposure to an actual forest setting or a nature-derived olfactory stimulation. Similar results were obtained in this study, thus clarifying the relaxation effects of nature-derived 3D images compared with those of similar 2D images. Our previous study demonstrated potential differences in physiological responses to natural and projected scenes by measuring activation of the prefrontal cortex in response to the presentation of actual foliage plants and projected images of the same plants [32]. However, no studies have evaluated differences in physiological effects between 3D and 2D plant images. The left and right prefrontal cortices differentially responded to the 3D image in this study. Oxy-Hb concentration measured by NIRS significantly decreased in the right hemisphere, but not in the left hemisphere, while there was no significant hemispheric difference in response to the 2D image.

Homae [33] highlighted the functional organization of the two hemispheres by reviewing some of the most recent functional NIRS studies that reported hemispheric differences in activation patterns. Most NIRS studies of visual stimuli have reported unilateral activation (significant activation in one hemisphere only), in accord with our current results. Further study on hemispheric differences in NIRS activity during sensory stimulation is required to reveal the functional implications of this lateralization for the analysis of different sensory modalities.

Some researchers have clarified the relationship between brain activity and neurotransmitters using primates in relation to the correlations of prefrontal cortex activity and the reward or reward anticipation [34-37]. In contrast, NIRS, used in the present study, cannot identify a brain region with high resolution; therefore, future studies involving different methods may shed light on the topic mentioned above. It is considered that NIRS reflects the activity of the prefrontal cortex, but cannot characterize brain activity in greater detail.

In conclusion, visual stimulation by a realistic 3D image of nature (water lily) induced a significant decrease in right prefrontal cortex activity, while a similar 2D image did not. Moreover, the 3D image resulted in a greater decrease in sympathetic nerve activity, consistent with greater physiological relaxation. However, it should be emphasized that these results were obtained only in males within a narrow age range, and functional lateralization is known to vary by age [33]. Therefore, additional studies in other age groups and mixed male–female populations are necessary to determine the generality of the greater physiological relaxation effects of 3D images of nature.

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Figure

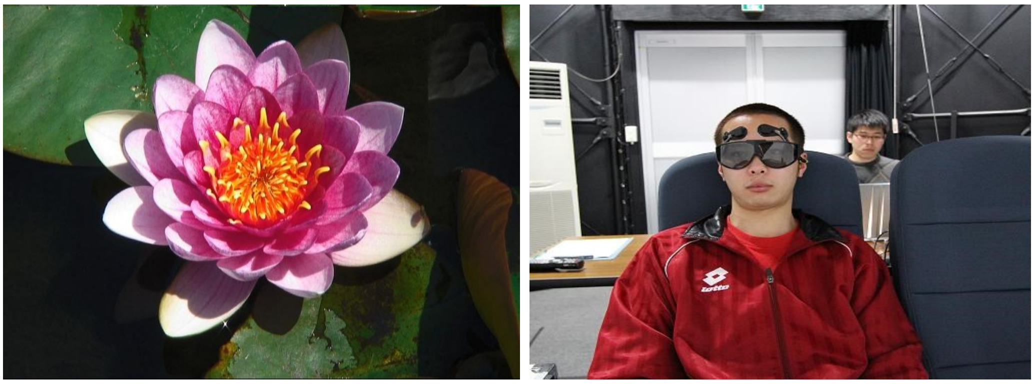


Fig. 1. The visual stimulus used (2D and 3D images of a water lily, left) and the scene during visual stimulation (right)

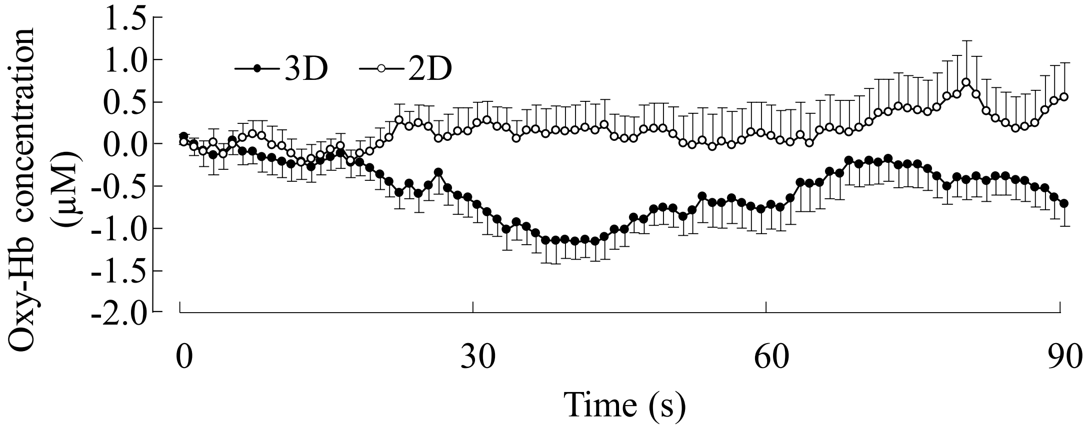


Fig. 2. Changes in oxy-Hb concentration in the right prefrontal cortex while viewing a 3D image or a similar 2D image

Measurements were acquired at 1 Hz using near infrared spectroscopy (NIRS)

The oxy-Hb concentration shown is the difference between the stimulus condition value at time “t” and the mean pre-stimulus value

Data are expressed as mean ± SE; n = 14

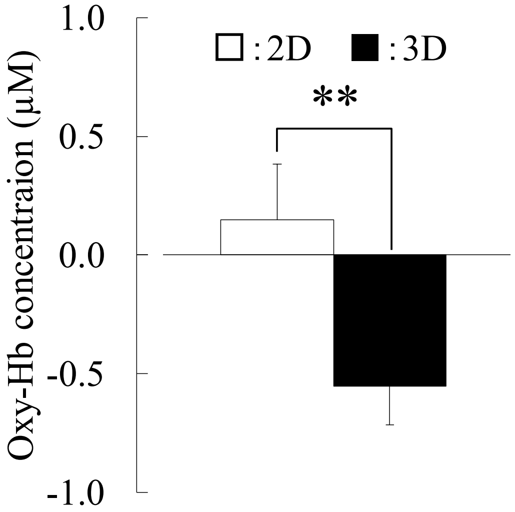


Fig. 3. Comparison of the mean oxy-Hb concentration in the right prefrontal cortex during the 90-s viewing period for the 3D and similar 2D image

The oxy-Hb concentration shown is the difference between the mean stimulus condition value and mean pre-stimulus value. Data are expressed as mean ± SE; n = 14

**p < 0.01 by the paired t-test (one-sided)

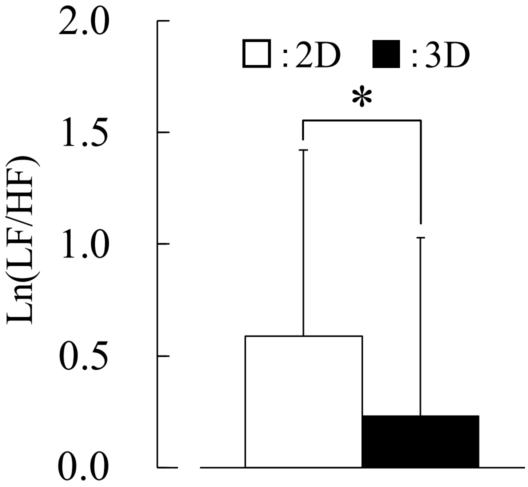


Fig. 4. Comparison of the [ln(LF/HF)ratio] during visual stimulation by a 3D or a similar 2D image

Data are expressed as mean ± SD; n = 17

*p < 0.05 by paired t-test (one-sided)

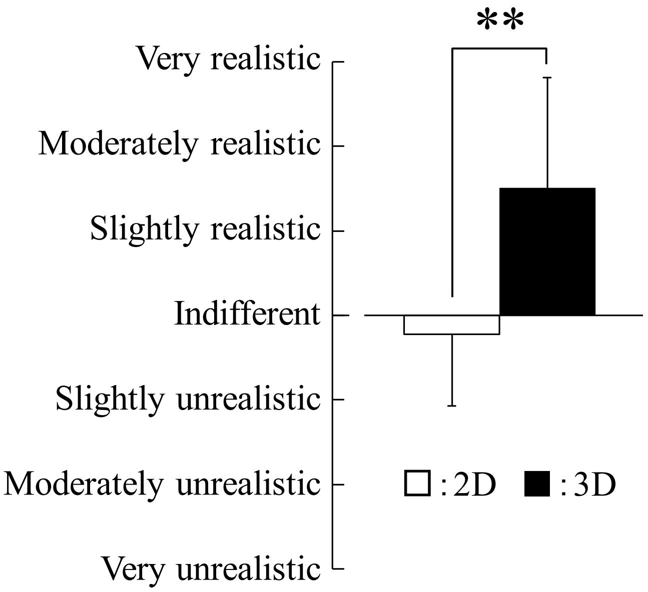


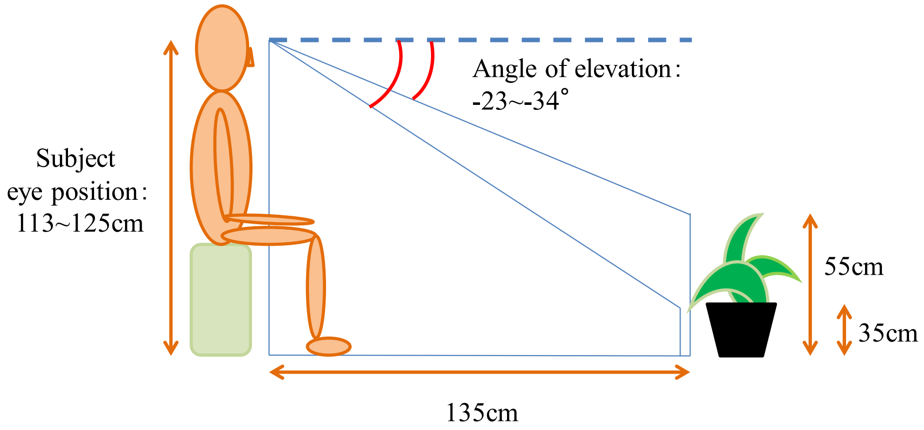
Fig. 5. Subjective evaluation of realistic feelings experienced during visual presentation of a 3D image or similar 2D image using a modified semantic differential (SD) method

Data are expressed as mean ± SD; n = 11

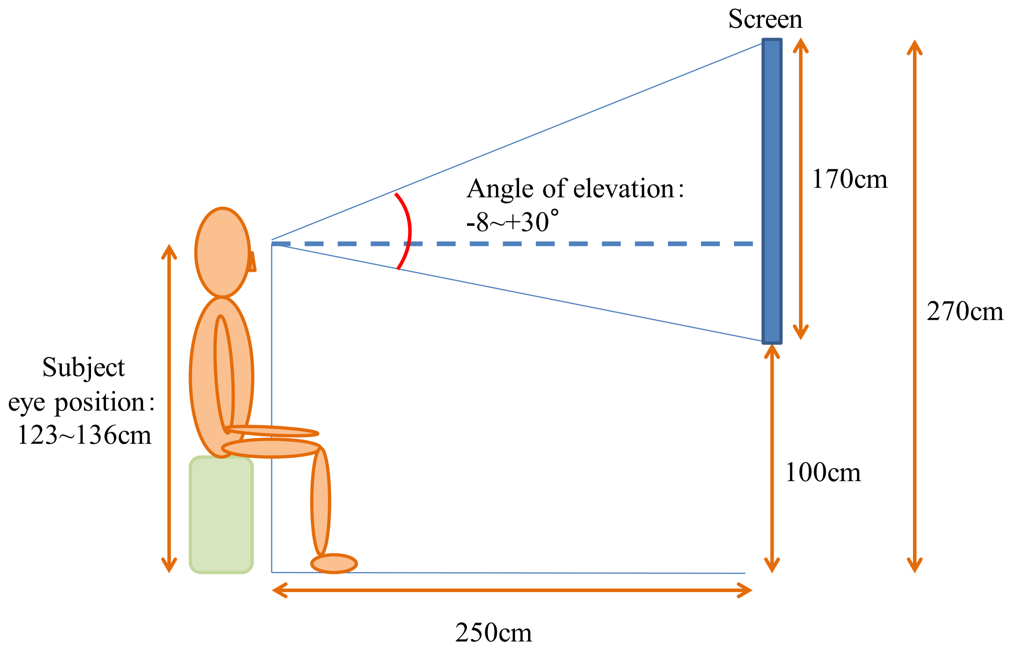
**p < 0.01 by Wilcoxon signed-rank test (one-sided)

4.4. Appendix of visual stimulation

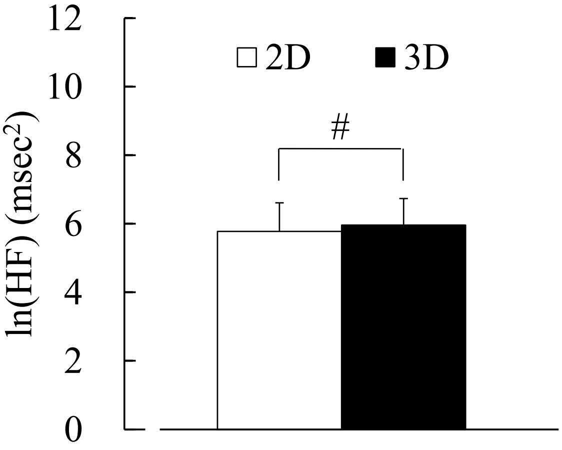
A schematic of the experimental setup in chapter 4.2 is shown below.



A schematic of the experimental setup in chapter 4.3 is shown below.



In chapter 4.3., we studied visual stimulation using the azalea rather than the water lily. The 3D images of azaleas induced a marginally significant increase ln(HF), which reflects parasympathetic nerve activities compared with the 2D images of azaleas. The visual-stimulation results from azaleas are as follows;



Comparison of the ln(HF) during visual stimulation by a 3D or a similar 2D image of azaleas

Data are expressed as mean ± SD; n = 16

#p < 0.10 by paired t-test (one-sided)

4.5. Conclusion of visual stimulation

4.2. Effect of stimulation by foliage plant display images on prefrontal cortex activity: a comparison with stimulation using actual foliage plants

Compared with a projected image of foliage plants, viewing actual foliage plants significantly increased oxy-Hb concentrations in the prefrontal cortex.

4.3. Effects of stimulation by three-dimensional natural images on prefrontal cortex and autonomic nerve activity: a comparison with stimulation using two-dimensional images

Compared with visual stimulation by 2D images, that by 3D images resulted in a significant decrease in oxy-Hb concentration in the right prefrontal cortex and lower sympathetic activity, as calculated by the ratio of the low frequency (LF) to high frequency (HF) HRV component. Visual stimulation by realistic 3D floral images promotes physiological relaxation more effectively than the corresponding 2D image.

The comparison between the physiological responses of visual stimulations from images of foliage plants with actual foliage plants revealed that the actual foliage plants increased the brains' prefrontal cortex activity more than the display images. We then compared the physiological responses to visual stimulations using various 3D plant images and 2D images. Our findings indicated that the 3D images of the water lily decreased the brains' prefrontal cortex activity and sympathetic nerve activity, clearly indicating that the plant-derived stimulation had physiological relaxation effects.

The above experiments that utilized display and 2D images as a control did not change in each physiological index. In this study, we used foliage plants and flowers, and it may be assumed that we cannot compare them simply because objects and subjects were different. However, we propose the following explanations. The actual visual stimulation was thought to act as an active stimulation to bring activation of the brain's prefrontal cortex activity, and the 3D visual stimulation acted as a passive stimulation to cause a calming effect on the brain's prefrontal cortex activity. Although the results of 3D visual stimuli decreasing brain activity in the right hemisphere but not left hemisphere are interesting, further study is required.

5.Conclusion

The purpose of this study was to characterize the physiological effects of empirically studied plant-derived stimulation. As part of the scope of this field, we performed a study using the plant, perilla. The literature review indicated that there was no evaluation data describing human physiological response to olfactory stimulation. Furthermore, we found very few physiological data describing the response to plant-derived olfactory or visual stimulation, clearly indicating the necessity for data collection.

Therefore, we performed independent stimulation studies as indoor experiments to characterize the physiological effects of plant-derived olfactory or visual stimulation.

To characterize the physiological responses to plant-derived olfactory stimulation in an olfactory-stimulation experiment, we used perilla, rose, and orange essential oils and fresh rose flowers. Our results confirmed that olfactory stimulation with perilla, rose, and orange essential oils calmed the brain’s prefrontal cortex activities, while the olfactory stimulation using fresh rose flowers increased parasympathetic nerve activity. However, the finding that rose and orange essential oil decreased brain activity in the right but not in the left hemisphere was very interesting. Considerable information about the brain activity of olfactory stimuli remains unknown and requires further study. Plant-derived olfactory stimulation clearly provided physiological relaxation effects.

Comparison between the physiological responses to visual stimulation using display images of foliage plants and actual foliage plants in visual stimulation experiments revealed that the actual foliage plants increased the brain’s prefrontal cortex activity more than that with display images. Comparison of the physiological responses to visual stimulation with various 3D plant images and 2D images showed that 3D images of water lily decreased the brain’s prefrontal cortex activity and sympathetic nerve activity. Thus, plant-derived stimulation clearly has physiological relaxation effects.

In this study, we used foliage plants and flowers, and it may be assumed that we cannot compare them simply because objects and subjects were different. However, we propose the following explanations. The display and 2D images used in these experiments as a control did not change in each physiological index. Actual visual stimulation acted as active stimulation to activate the brain’s prefrontal cortex, and 3D visual stimulation acted as passive stimulation to exert a calming effect on the brain’s prefrontal cortex activity. Although the results of 3D visual stimuli decreasing brain activity in the right hemisphere but not left hemisphere are interesting, further study is required.

In this study, we scientifically elucidated the physiological responses to plant-derived stimulation from the viewpoint of EBM. These findings will contribute to improving the QOL experienced in artificial environments.

6. Challenges and Future Steps

Below, we discuss challenges arising from this study.

First, although we examined the effect of essential oils on brain activity and fresh flowers on autonomic nerve activity through an olfactory stimulation study, more comprehensive studies using a superior physiological evaluation system are required for studying brain, autonomic nerve, endocrine, and immune activities.

Second, in this study, we performed olfactory and visual stimulation experiments independently. However, combined olfactory and visual stimulation experiments are required.

Third, interpretation of changes in the oxy-Hb concentration revealed by brain activity measurements is necessary. Further research is required to compare the effects of conventional relaxation methods with those reviewed in this study. Therefore, this future step would help in understanding the oxy-Hb concentration changes observed in this study.

Fourth, further research is required on changes in prefrontal cortex laterality.

This study is important because it provides data on physiological changes occurring during nature-derived stimulation, which extremely few studies have investigated thus far. Next steps should focus on two particular challenges discussed above.

First, comprehensive physiological studies including measurement of brain activity by near-infrared time-resolved spectroscopy, autonomic nerve activity by heart rate variability, endocrine activity by salivary stress hormone (e.g., cortisol) levels, and immune activity by natural killer cell activity should be conducted.

Second, combined olfactory and visual stimulation studies should be conducted.

At present, very little data is available in this field. We believe this research, and the results described above, will considerably contribute to this academic field.

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