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The Sleep-Enhancing Effect of Valerian Inhalation and Sleep-Shortening Effect of Lemon Inhalation

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Abstract

We examined the effects of odorant inhalation on the sleep–wake states in rats. Odorants used in the experiment were clove, jasmine, lavender, lemon, peppermint, pine, rose, sandalwood, valerian, and ylang-ylang. Valerian and rose inhalation significantly prolonged the pentobarbital-induced sleeping time, whereas lemon inhalation significantly shortened it. The effect of valerian inhalation was markedly noticeable. In the anosmic rats, a significant effect of odorants on the pentobarbital sleep time was not seen. Electroencephalographic studies on natural sleep revealed that rose inhalation did not exert any significant effect on sleep, but a significant shortening in sleep latency and a significant prolonging in total sleep time were observed with valerian inhalation, whereas a significant prolonging in sleep latency was observed with lemon inhalation. Such effects of valerian and lemon inhalation were not admitted in anosmic rats. γ-Aminobutyric acid (GABA) transaminase assay indicates that valerian inhalation decreases the activity of the enzyme and enhances GABA activity. Although valerian has been reported to exert a good effect for sleep as a medicine for internal use, the present study is the first medical report suggesting that the inhalation of valerian may enhance the sleep. On the other hand, the present results may suggest the possibility that lemon inhalation may cause a worsening of insomnia symptoms.

Key words: anosmia, EEG, GABA transaminase, lemon, pentobarbital sleep time, valerian

Introduction

Extracts of numerous plants are traditionally used to relieve anxiety, restlessness, insomnia, etc. The olfactory input has been shown to have the ability to stimulate, suppress, or modulate various types of behavior related to the central nervous system (CNS) in mammalian species (Alberts and Galef 1971; Leonard and Tuite 1981). Such as drugs that act on CNS, odorants capable of influencing CNS may be classified as CNS depressants or CNS stimulants, depending on their most prominent effect of postulated medical application. The papers on psychotropic effects of odorants, however, have been only seen here and there, although variety is said to exist in the aromatherapy based on both the legend and the experience. The effects of odorants on the brain function have been studied by using the alpha and delta activities in the electroencephalogram (EEG) (Lorig and Schwarts 1988) or contingent negative variation (Torii et al. 1988; Manley 1993), and these studies have shown that some odorants exert stimulant or inhibitory effects on the brain function. We previously reported that the inhalation of lemon significantly reduced the total immobility time and potentiated the effect of tricyclic antidepressant imipramine on total immobility time in the forced swimming test in rats (Komori et al. 1995). We also reported that the inhalation of lemon exerted antistress effects, probably via the interaction among the nervous, endocrine, and immune systems, in rats using immune parameters as indices (Fujiwara et al. 1998). On the other hand, Koo et al. (2003) reported CNS inhibitory effects of odorant inhalation of essential oil from Acori graminei Rhizoma. In addition, to date, only a few papers have reported on sedative or activating properties of some essential oils on animals after odorant inhalation under standardized experimental procedures.

The problem of excessive use of benzodiazepine hypnotics has been described in recent years, and alternatives to hypnotic benzodiazepines such as cognitive therapy (Hackmann 1993) and relaxation (Sloan et al. 1993) are applicable only under limited conditions. We consider that the application of odorants may be useful for keeping sound sleep and decreasing the use of hypnotics. For the effects of odorants on sleep, Tsuchiya et al. (1991) reported the effect of various odorants

on pentobarbital-induced sleep time. The sleep time induced by pentobarbital administration was capable of being affected by odorant inhalation, and the pattern of the effect differed depending on the odorants. The reported effects of some odorants, however, were not fully appropriate for clinical application. In a preliminary study, the inhalation of valerian was found to markedly prolong the pentobarbital-induced sleep time in rats, and therefore, we planned the present study. The pentobarbital-induced sleep, however, is anesthesia, which is different from natural sleep, and therefore, we also examined the effects of odorants on natural sleep using EEG in this study. Moreover, to clarify the mechanism of effects of valerian inhalation, we performed a γ-aminobutyric acid (GABA) transaminase assay using rat brain specimens obtained after valerian inhalation.

Materials and methods

Animals

Male Wistar rats, 12 weeks old, were purchased from SLC (Shizuoka, Japan) and then were used throughout the experiments. All rats were housed in cages in a quiet room and given access to pelleted diet and water ad libitum. The room temperature was controlled (20 ± 2 °C), and lights were on between 06:00 AM and 06:00 PM. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Mie University Graduate School of Medicine.

Apparatus and exposure to odorants

The experiment was performed using 10 kinds of odorants: clove, jasmine, lavender, lemon, peppermint, pine, rose, sandalwood, valerian, and ylang-ylang. Table 1 shows the details of the odorants. These odorants were freely available commercial products and purchased from Shiseido (Tokyo, Japan).

The apparatus for odorant application consisted of an air pump, flow meter, glass tube covered with a mantle heater (maintained at approximately 100 °C), a chamber (height 14 cm, diameter 25 cm), flow meter, and an air pump, as described previously (Komori et al. 2003). All these components were connected by tube in the above order. One rat was placed in the chamber. Triethyl citrate was used as solvent for dilution, and 10% solution of odorant was used in the experiment. For odorant application, the volatile odorant liquid was injected into the glass tube at 0.1 ml/h using an infusion pump. The chamber was provided with 2 holes. One hole was connected to the glass tube, and the odorized air was delivered into the chamber by driving air through the glass tube. The flow rate was maintained at 2.0 l/min using a flow meter. Through the other hole, an equal volume of air was exhausted by the driving air. The flow rate was also maintained at 2.0 l/min using a flow meter. The control rats were exposed to pure air in the same chamber as described above.

Table 1 Details of the odorants used in the experiment

Materials	Species	Parts	Production methods
Lemon oil	*Citrus limonum*	Peel	Cold pressed and deterpenized
Jasmine absolute	*Jasminum officinale* var. *grandiflorum*	Flower	Extraction in the enfleurage
Clove oil	*Eugenia caryophyllata*	Bud	Steam distillation
Peppermint oil	*Mentha piperita*	Herb	Steam distillation
Lavender oil	*Lavandula officinalis*	Herb	Steam distillation
Ylang-ylang oil	*Cananga odorata*	Flower	Steam distillation
Sandalwood oil	*Santalum album*	Wood	Steam distillation
Pine oil	*Pinus palustris*	Needle	Steam distillation
Rose oil	*Rosa damacena*	Flower	Steam distillation
Valerian oil	*Valeriana officinalis*	Root	Steam distillation

Pentobarbital sleeping time in rats

Pentobarbital sodium (Nembutal, Dainippon Pharmaceutical Co., Tokyo, Japan) was diluted in physiological saline and administered to each rat intraperitoneally (i.p.) at a dose of 50 mg/kg to induce sleep. The anesthetized rat was then placed on its back in the same chamber from which it came before the administration of pentobarbital. The sleep time of the individual rats was measured to the nearest 1 min. With the criterion for sleep being loss of righting reflex (Rolland et al. 1991), the sleep time was defined as the time elapsed between the i.p. administration of pentobarbital and the first time that the animal spontaneously righted itself. Sleeping time was recorded from the disappearance of the righting reflex until its recovery and compared with those of the control group. As a positive control, chlorpromazine (CP) hydrochloride was administered at 10 mg/kg per os 30 min prior to administration of pentobarbital.

Producing anosmic rats

A modification of the technique described previously (Alberts and Galef 1971) was used to introduce peripheral anosmia. One hundred and sixty rats were assigned at random to the zinc sulfate treatment group, and they were given a single intranasal treatment with zinc sulfate. The olfactory intact group consisted of 150 rats that received an intranasal treatment with physiological saline. The intranasal treatment was performed under ether anesthesia. The zinc sulfate solution used was isotonic with normal body fluids (7.65%).

A 1-in. × 25-ga blunted hydrodermic needle was inserted into the external nares so that the tip entered the nasal cavity. Approximately 50 µl of zinc sulfate solution was then flushed through the syringe. Mild suction was immediately applied to the external nares as soon as the solution appeared, and the oral cavity was aspirated to remove saliva and excess solution with a blunt 25-ga needle connected by tubing to

a suction pump. The rat was then returned to its home cage. The rats were housed and fed the same as for the preceding animals for another 9 days. Control rats were treated identically, except that physiological saline was substituted for the zinc sulfate solution.

Seven days after the intranasal treatment, all rats were tested for olfactory sensitivity by means of a cotton swab impregnated with Vintage cologne (Shiseido) placed in front of the individual animal's nose while it was alone in the cage. Two naive observers then judged whether the animal could smell it based on the criterion or whether he avoided the swab, ignored it, or chewed on it (Hull et al. 1974).

Rats were subjected to the olfactory sensitivity test 2 days before the experiment. A total of 139 rats, which ignored the swab impregnated with cologne after zinc sulfate intranasal treatment, were defined as anosmic. One hundred and thirty-six rats avoided the swab after saline treatment and were defined as the olfactory intact group. One hundred and thirty anosmic and olfactory intact rats were used in the experiment.

EEG recordings

The previous report (Shinomiya et al. 2005) was referred to for EEG studies on the effects of odorants on natural sleep. All rats were chronically implanted with EEG, electromyogram (EMG), and electrooculogram (EOG) electrodes, for the assessment of sleep–wake states, under pentobarbital sodium anesthesia (40 mg/kg body weight, i.p.). For EEG recording, a stainless steel screw electrode was chronically implanted into the right frontal cortex. EOG was recorded with 2 electrodes slipped under the eyelid. To record EMG, we implanted stainless steel wire electrodes into the dorsal neck muscle. A stainless steel screw fixed in the left frontal bone served as the reference electrode. The electrodes were soldered to a plug that was fixed to the skull with dental cement. At least 7 days were allowed to pass for the rats to recover from the surgery.

The rats, with chronically implanted electrodes, were placed in the chamber (height 14 cm, diameter 25 cm) in which they could move freely. The rats were put in the chamber 1 day prior to the recording to habituate them to the surroundings. EEG, EMG, and EOG were recorded from 09:00 AM to 03:00 PM. The sleep–wake states were automatically classified by 10-s epochs as wakefulness, non–rapid eye movement (non-REM), or REM sleep (by SleepSign for animal version 2.0, Kissei Comtec America, Inc., Irvine, CA) according to the criteria previously described (Shinomiya et al. 2003; Shigemoto et al. 2004). Sleep latency was defined as time elapsed between the application of odorized or pure air in the chamber and the first non-REM sleep episode lasting at least 1 min (Vyazovskiy et al. 2005).

Experiment 1

In experiment 1, the pentobarbital sleep time was measured using 10 kinds of odorants: clove, jasmine, lavender, lemon, peppermint, pine, rose, sandalwood, valerian, and ylang-ylang. The rats were exposed to each type of either pure air (the control and the positive control rats) or odorized air 1 h before pentobarbital administration, followed by exposure to each type of pure air or odorized air until the appearance of righting reflex. The positive control rats were given CP at 10 mg/kg per os 30 min prior to pentobarbital administration. Each rat was used only once. In each group, $n = 10$, and total number of rats used was 120.

Experiment 2

The pentobarbital sleep time was measured using anosmic rats. Lemon, rose, and valerian, which all demonstrated a significant effect in experiment 1, were used. The pentobarbital sleep time of the following 12 groups, normal control group, positive control group, pure air–inhaled olfactory intact animal group, pure air–inhaled olfactory intact positive control group, odorant-inhaled olfactory intact animal group (odorant: lemon, rose, or valerian), pure air–inhaled anosmic animal group, pure air–inhaled anosmic positive control group, odorant-inhaled anosmic animal group (odorant: lemon, rose, or valerian), were measured. The methods of odorant exposure and CP and pentobarbital administration were the same as in experiment 1. The normal control rats were exposed to pure air before and after pentobarbital administration. Each rat was used only once. In each group, $n = 10$, and total number of rats used was 120.

Experiment 3

In experiment 3, an EEG study was performed on 3 kinds of odorants (lemon, rose, and valerian) whose inhalation was shown to affect significantly the pentobarbital-induced sleeping time in experiment 1. The rats, with chronically implanted electrodes, were placed in the chamber (height 14 cm, diameter 25 cm) in which the rats inhaled either odorized or pure air from 09:00 AM to 03:00 PM. The EEG recordings were performed from 09:00 AM to 03:00 PM. As a positive control, CP was administered at 10 mg/kg per os at 08:30 AM. Each rat was used only once. Normal control, positive control, and each odorant group were $n = 10$, and the total number of rats used was 50.

Experiment 4

The experiment 4 was performed using lemon and valerian that significantly affected the natural sleep in experiment 3. Using the olfactory intact rats and the anosmic rats, EEG studies were performed in the same manner as for experiment 3. Each rat was used only once. Normal control group, positive control group, pure air–inhaled olfactory intact group, pure air–inhaled olfactory intact positive control group, odorant-inhaled olfactory intact group (odorant: lemon or valerian), pure air–inhaled anosmic group, pure air–inhaled anosmic positive control group, odorant-inhaled anosmic

group (odorant: lemon or valerian) were $n = 10$, and total number of rats used was 100.

Experiment 5: in vivo GABA transaminase assay

The rats were placed in the chamber (height 14 cm, diameter 25 cm) in which they inhaled either odorized or pure air from 09:00 AM to 12:00 PM. The olfactory intact rats and anosmic rats were used, and valerian, rose, and lemon were used as the odorants. The control group was not treated at all. Each rat was used only once. In each group, $n = 10$, and total number of rats used was 90. Next, the whole brain was isolated and then homogenized with a glass homogenizer of 0.1 M potassium phosphate (KP) buffer (pH = 7.4). Homogenates were centrifuged in 600 × g for 10 min at 4 °C, and the supernatant was collected and recentrifuged in 10,000 × g for 20 min at 4 °C. Postmitochondrial fractions were ultracentrifuged in 105,000 × g for 1 h, and the supernatant was used as an enzymatic source for the GABA transaminase assay as described previously (Koo et al. 2003). GABA, α-ketoglutaric acid, 0.15 M KP buffer (pH = 8.0), and tissue homogenates were incubated at 37 °C for 30 min, followed by the addition of nicotinamide adenine dinucleotide ($NADP^+$). The amount of nicotinamide adenine dinucleotide phosphate (NADPH) generated in the brain tissue for 20 min was measured by spectrophotometer at 340 nm as an activity of GABA transaminase.

Statistical analysis

In all experiments, a statistical analysis was performed using Student's *t*-test compared with the control, and statistical significance was recognized when the *P* value was <0.05.

Results

Table 2 shows the results of experiment 1. The odorants which altered the pentobarbital sleep time by more than 10% of the control were lemon (−22%), sandalwood (+11%), pine (+12%), rose (+15%), and valerian (+46%). A positive control showed a +131% prolongation in comparison with the normal control. Student's *t*-test revealed that the effects of lemon, rose, and valerian reached statistical significance ($P < 0.05$).

Table 3 shows the comparative effects of odorant inhalation on the pentobarbital sleep time in anosmic rats and olfactory intact rats. The pentobarbital sleep time in pure air–inhaled olfactory intact rats and anosmic rats was not significantly different from the normal control. In addition, the CP-treated rats in the olfactory intact and anosmic rats were not significantly different from the positive control. A clear difference between the anosmic rats and the olfactory intact rats was observed in the effects of odorant inhalation on the pentobarbital sleep time. The pentobarbital sleep time was affected by the odorant inhalation in the olfactory intact rats, namely, the sleep time was shortened by 20% for lemon, and it was prolonged 14% by rose and 41% by valerian inhalation. These results were similar to the results obtained in the experiment 1. However, in the anosmic rats, no significant effect on the pentobarbital sleep time was seen with lemon, rose, or valerian.

Table 2 Effects of the inhalation of various odorants on the pentobarbital sleep time in rats

Odorant	Pentobarbital sleep time (min)	Control (%)
Control	66.7 ± 4	
CP	154.4 ± 5	+131*
Lemon	52.3 ± 3	−22*
Jasmine	63.2 ± 5	−5
Clove	64.0 ± 4	−4
Peppermint	69.8 ± 4	+5
Lavender	70.7 ± 5	+6
Ylang-ylang	72.6 ± 4	+9
Sandalwood	74.1 ± 5	+11
Pine	74.7 ± 5	+12
Rose	76.5 ± 6	+15*
Valerian	97.4 ± 8	+46*

The data show the mean ± standard deviation. For all data, $n = 10$. CP (10 mg/kg) was administered per os.*Significantly differs from the control at $P < 0.05$.

Table 3 Effects of the inhalation of various odorants on the pentobarbital sleep time in olfactory intact and anosmic rats

Group	Pentobarbital sleep time (min)	Control (%)
Normal control	67.9 ± 4	
CP	159.8 ± 5	+135*
Olfactory intact rats		
Pure air	64.3 ± 4	−5
CP	152.6 ± 6	+125*
Lemon	54.2 ± 3	−20*
Rose	77.3 ± 6	+14*
Valerian	95.8 ± 8	+41*
Anosmic rats		
Pure air	69.2 ± 4	+2
CP	151.2 ± 5	+123*
Lemon	64.5 ± 3	−5
Rose	71.1 ± 4	+5
Valerian	69.6 ± 4	+3

The data show the mean ± standard deviation. For all data, $n = 10$. CP (10 mg/kg) was administered per os.*Significantly differs from the control at $P < 0.05$.

Figure 1 shows the results of sleep latency in experiments 3 and 4, and Figure 2 shows the results of total sleep time and the sleep–wake states in experiments 3 and 4. A positive control showed a significant shortening in sleep latency by 78% in experiment 3 and by 81% in experiment 4. In experiments 3 and 4, a positive control showed a significant prolongation in total sleep time, whereas no significant difference was found in non-REM and REM sleep time in comparison with the normal control. In experiment 4, the olfactory intact pure air–inhaled rats and the anosmic pure air–inhaled rats were not significantly different from the normal control, and the CP-treated olfactory intact rats and the CP-treated anosmic rats were not significantly different from the positive control. These results were omitted in Figures 1 and 2. Valerian inhalation significantly shortened the sleep latency (−34%), whereas lemon inhalation significantly prolonged it (+27%) (Figure 1). Valerian inhalation significantly prolonged the total sleep time (+18%), though lemon inhalation did not affect it (Figure 2). Rose inhalation did not affect the sleep latency or the total sleep time (Figure 1). The ratio of non-REM sleep time to REM sleep time was not affected by any odorants (Figure 2).

The production of NADPH (nmol/mg protein/h) by GABA transaminase was shown in Table 4. In the olfactory intact rats, the production of NADPH in pure air inhalation was not significantly different from that in the normal control. The production of NADPH was significantly lower in valerian inhalation than in the control, whereas lemon and rose inhalation did not affect the production of NADPH. On the other hand, no changes in the GABA transaminase activity based on any odorant inhalation were found in the anosmic rats.

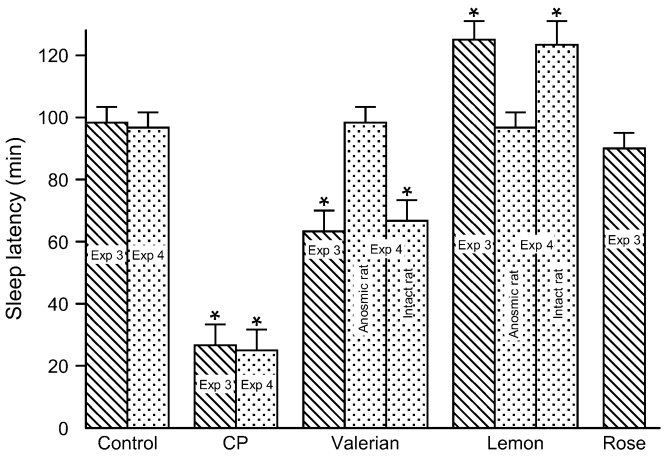

Figure 1 Effects of odorant inhalation on sleep latency in normal and anosmic rats. The columns and vertical bars represent the means ± standard deviation. For all data, n = 10. CP (10 mg/kg) was administered per os. *, Significantly differs from the control at $P < 0.05$.

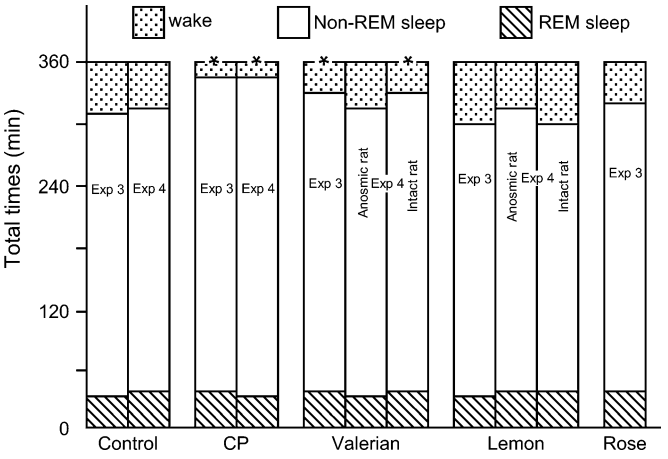

Figure 2 Effects of odorant inhalation on the total time of each sleep state in normal and anosmic rats. Columns and vertical bars represent the means ± standard deviation. For all data, n = 10. CP (10 mg/kg) was administered per os. *, Significantly differs from the control at $P < 0.05$.

Discussion

In the present study, valerian and rose inhalation were found to significantly prolong the pentobarbital sleep time, whereas lemon inhalation significantly shortened it. In particular, valerian's effect was marked. Though the increase and decrease of the pentobarbital-induced sleep time is a method that is useful for examining the stimulatory or inhibitory effects on CNS, pentobarbital sleep is anesthetizing but not a natural sleep. GABA is the major fast inhibitory neurotransmitter in the CNS, and pentobarbital is well known to potentiate the effects of GABA, acting at their own receptor sites on the GABA/receptor ionophore complex (Olsen 1981; Ticku and Maksay 1984). Although the factors that may affect the depth of anesthetizing are known to be pregnancy, age, low O_2, high CO_2, body temperature, and the mass of muscle and fat, all rats were used under the same condition in the present studies, and there is no factor affecting the present results. There is a common mechanism to natural sleep and anesthetizing, but anesthetizing is known to inhibit the REM sleep, various reflexes, and senses, and it is different from that for natural sleep. We therefore studied the effects of odorants on natural sleep. Although rose inhalation did not exert any significant effect, lemon inhalation prolonged the sleep latency, whereas valerian inhalation shortened the sleep latency and prolonged the total sleep time. No such effects of lemon and valerian were found in anosmic rats as in the case of pentobarbital-induced sleep, and therefore, the effects were thought to be through the olfactory system.

Volatile substances affect animals through 3 different pathways, the absorption from the mucous membranes of nose and respiratory tract, the stimulation of bronchial and pulmonal chemical receptors, and the olfactory cells as odor. Especially, odorant substances can move into the circulating blood, and it may give rise to a controversy in the effects of odorants. Aoshima and colleagues (Aoshima and Hamamoto 1999; Hossain et al. 2002, 2004) have suggested that odorant compounds are absorbed into the blood and carried to

Table 4 Effects of odorant inhalation on the brain GABA transaminase activity in vivo

	NADPH (nmol/mg protein/min)
Normal control	1.27 ± 0.08
Olfactory intact rats	
Pure air	1.22 ± 0.07
Lemon	1.19 ± 0.08
Rose	1.13 ± 0.05
Valerian	0.78 ± 0.06*
Anosmic rats	
Pure air	1.24 ± 0.07
Lemon	1.18 ± 0.05
Rose	1.21 ± 0.05
Valerian	1.16 ± 0.07

The data show the mean ± standard deviation. For all data, $n = 10$.
*Significantly differs from the control at $P < 0.05$.

the brain through the blood–brain barrier and then affect the $GABA_A$ receptor–mediated response which would have a tranquilizing effect on the brain, although the possibility cannot be ruled out that odorant compounds affect pentobarbital decomposition in the liver. The present results using anosmic rats, however, indicated that olfaction is essential for the effect on pentobarbital sleep time by the inhalation of valerian, lemon, and rose. Koo et al. (2003) found that inhalation of the essential oil of Acori graminei Rhizoma progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened, and the inhalation of this odorant also inhibited GABA transaminase, a degrading enzyme for GABA, as the inhalation period was lengthened. In the present study, we also performed a GABA transaminase assay, and the results indicated that valerian inhalation induced a decrease in the enzyme activity.

Valerian (*Valeriana officinalis*) is well summarized in the review (Plushner 2000). Valerian is a flowering herb native to North America, Europe, and Asia but now grown in most parts of the world. Of approximately 200 known species, *V. officinalis* is the one most commonly used for medicinal purposes (Leathwood et al. 1982; Hobbs 1989). Valerian has been used as a sedative since it was described by the ancient Greeks and Romans (Houghton 1988). The use of valerian to treat insomnia and nervous conditions began in the late 16th century and was firmly established by the 18th century (Hobbs 1989), and the current popular interest in valerian is primarily related to its effects on sleep. Today, *V. officinalis* is still included in German, Swiss, British, French, Japanese, Chinese, and European pharmacopoeias. Several reports suggest that valerian extracts reduced sleep latency and improves sleep structure and sleep perception of insomnia in healthy volunteers and patients suffering from sleep disorders (Leathwood et al. 1982; Kuhlmann et al. 1999; Herrera-Arellano et al. 2001; Cropley et al. 2002; Hallam et al. 2003; Fernandez et al. 2004).

Two constituents of valerian, namely, volatile oils and valepotriates, potentially account for its activity (Houghton 1999). The volatile oils are known for their characteristic strong odor and contain monoterpenes and sesquiterpenes (Houghton 1999). Monoterpenes are primarily borneol, and 3 major sesquiterpenes are valerenic acid, valeranone, and kessyl glycol. Valepotriates are esters of unsaturated terpene alcohols (Lindahl and Lindwall 1989). The purported mechanisms accounting for valerian's sedative–hypnotic effects include interaction with GABA receptors (Santos et al. 1994). An increase in the duration of sleep was induced by thiopental (Leuschner et al. 1993; Hiller and Zetler 1996). Recently, Shinomiya et al. (2005) reported that a significant shortening in sleep latency without any significant effects on the total times of wakefulness was observed with valerian extract per os. Although GABA is present in valerian extracts, its brain bioavailability via oral administration is uncertain (Santos et al. 1994). The action of valerian on the CNS might be due in part to GABA involvement through a number of mechanisms, including an inhibition of GABA uptake into synaptosomes (Santos et al. 1994). Valerian constituents inhibit the enzymatic breakdown of GABA and enhance benzodiazepine binding (Ortiz et al. 1999). The other potential mechanisms for the pharmacological activity of valerian have been proposed, including partial agonistic activities on 5-HT5a receptor (Dietz et al. 2005). The above-described reports on valerian, however, are in the case of a medicine for internal use.

The present results on lemon inhalation are consistent with the previous report (Tsuchiya et al. 1991). We cannot find out any other report on the relationship between lemon and sleep. The lemon odor is said to be useful in aromatherapy for improving concentration, and it is reported that lemon odor mitigated exhaustion and maintained vigor, although the existence or nonexistence of odor did not affect subjects' work efficacy (Kawamoto et al. 2005). We previously reported that a lemon odor has antistress (Fujiwara et al. 1998) and antidepressant effects (Komori et al. 1995). These results indicate that a lemon odor may therefore be a CNS stimulant. Regarding the clinical application of such odors for the treatment of depressive patients, we found that lemon odor can sometimes obstruct their sleep. In the treatment of depression, an improvement of sleep is important, and one must note the sleep-shortening effect of lemon odor.

Although the mechanism by which the olfactory stimulation produces its effect on the sleep is currently unclear and further studies are needed, the present results may provide useful information that can lead to the clinical application of odorants in the future.

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