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Effect of ''Rose Essential Oil'' Inhalation on Stress-Induced Skin-Barrier Disruption in Rats and Humans

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Abstract

In stressed animals, several brain regions (e.g., hypothalamic paraventricular nucleus [PVN]) exhibit neuronal activation, which increases plasma adrenocorticotropic hormone (ACTH) and glucocorticoids. We previously reported that so-called "green odor" inhibits stress-induced activation of the hypothalamo–pituitary–adrenocortical axis (HPA axis) and thereby prevents the chronic stress-induced disruption of the skin barrier. Here, we investigated whether rose essential oil, another sedative odorant, inhibits the stress-induced 1) increases in PVN neuronal activity in rats and plasma glucocorticoids (corticosterone [CORT] in rats and cortisol in humans) and 2) skin-barrier disruption in rats and humans. The results showed that in rats subjected to acute restraint stress, rose essential oil inhalation significantly inhibited the increase in plasma CORT and reduced the increases in the number of c-Fos-positive cells in PVN. Inhalation of rose essential oil significantly inhibited the following effects of chronic stress: 1) the elevation of transepidermal water loss (TEWL), an index of the disruption of skin-barrier function, in both rats and humans and 2) the increase in the salivary concentration of cortisol in humans. These results suggest that in rats and humans, chronic stress-induced disruption of the skin barrier can be limited or prevented by rose essential oil inhalation, possibly through its inhibitory effect on the HPA axis.

Key words: corticosterone, cortisol, rose essential oil, skin barrier, stress

Introduction

It is well known that "acute" exposure to stressful stimuli results in activation of the hypothalamo–pituitary–adrenocortical (HPA) axis and sympathetic nervous system and that this leads to stereotyped responses including increases in blood pressure and in the plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (Watanabe et al. 1998; DiMicco et al. 2006; Smith and Vale 2006). Moreover, it has been demonstrated that long-term activation of the HPA axis due to "chronic" stress has deleterious effects on various organs, including the skin (Arck et al. 2006; Fukada et al. 2007). Indeed, chronic stress reportedly induces skin-barrier disruption, as evidenced by an increase in transepidermal water loss (TEWL) in both mice (Aioi et al. 2001) and rats (Fukada et al. 2007).

A few years ago, Nakashima et al. (2004) found that in rats, inhalation of so-called green odor (extracted from green leaves) attenuated the acute stress-induced elevation in plasma ACTH. Incidentally, the term "green odor" is an idiomatic expression describing material consisting of a 50:50 mixture of *trans*-2-hexenal and *cis*-3-hexenol, not the odor itself. Since the term is already used in that way in the literature, it will be used here with the same meaning. Ito et al. (2009) found, also in rats, that green odor inhalation reduced the acute stress-induced increase in plasma CORT as well as the stress-induced activation of neurons located within the hypothalamic paraventricular nucleus (PVN). These results indicate that green odor may inhibit the activation of the HPA axis that occurs during acute stress. Meanwhile, we had shown that in rats, inhaling green odor has the ability to prevent the skin-barrier dysfunction induced by chronic stress (Fukada et al. 2007).

Rose oil from different sources has been examined for its relieving and sedative effects as well. For example, Hongratanaworakit (2009) found a relaxing effect of rose oil (derived from Rosa damascena Mill, Rosaceae) on humans after transdermal absorption. Recovery from "artificially" produced skin-barrier disruption, induced by tape

stripping, could reportedly be accelerated by inhalation of a component of Bulgarian rose oil, dimethoxymethylbenzene (Denda et al. 2000). In addition, de Almeida (2004) showed anxiolytic-like effects of a rose oil extracted from flowers of various rose species and purchased from Botica Ao Veado D'Ouro.

"Rose essential oil (essential oil derived from *Rosa alba*)," developed by Kanebo Cosmetics Inc., might therefore be expected to have a stress-relieving effect too. Indeed, we hypothesized that this rose essential oil would, like green odor, inhibit the acute stress-induced activation of the HPA axis and chronic stress-induced skin-barrier disruption.

To test this hypothesis, we examined the effects of rose essential oil inhalation 1) on the acute stress-induced increase in neural activity in PVN and in the plasma CORT level in rats, 2) on the chronic stress-induced skin-barrier disruption in human subjects and in rats, and 3) on the elevated salivary cortisol concentration in chronically stressed human subjects.

Materials and methods

Rat study

Animals

Male Wistar rats (11 weeks old) were housed in individual plastic cages (40 × 25 × 20 cm; length × width × depth) with wood chip bedding in a room maintained at 25 ± 1 °C, with the humidity set at 50%. They experienced a photoperiod of 12:12 h light:dark (lights on at 7:00 AM.). All had ad libitum access to drink (tap water) and standard laboratory rat chow. The protocols were reviewed by the Committee on the Ethics of Animal Experiments in Tottori University Faculty of Medicine, and the experiments were carried out in accordance both with the Guidelines for Animal Experiments at Tottori University Faculty of Medicine and with the Federal Law (no. 221) and Notification (no. 6) issued by the Japanese Government.

Rose essential oil

The odorant used was "essential oil of *R. alba*" (provided by Kanebo Cosmetics), which is obtained by hydrodistillation of rose flowers. Its characteristic components are citronellol (28.15%), geraniol (16.46%), nerol (8.65%), phenylethyl alcohol (4.84%), eugenol (1.10%), and linalool (1.38%). The oil was diluted with triethyl citrate to 3% (w/w). Triethyl citrate was used as vehicle.

Experimental protocols

Rats were restrained as required in a small cylindrical restrainer made of steel wire (7 × 22 cm [diameter × length]). Rose essential oil or its vehicle (i.e., triethyl citrate) was administered during the period of restraint (i.e., 90 min; see Measurement of plasma CORT). To this end, a cotton bowl (diameter = 15 mm) was impregnated with 0.2 mL of the rose essential oil or its vehicle, then held 3 cm from the nose of the rat. When rose essential oil was administered to freely moving rats, the same type of bowl was held in the restrainer mentioned above, and this was placed in one corner of the rat's "home-cage."

The rats were divided into the following 4 groups. In the vehicle + stress (V + S) and rose + stress (R + S) groups, vehicle or rose essential oil was administered during stress. In the control (C) group, nothing was administered at all to rats not subjected to restraint. The freely moving rats that inhaled rose essential oil for 90 min were classified as the rose (R) group. Because the restrained rats were unable to reach the food and water, all groups were deprived of food and water during the 90-min period of restraint or nonrestraint.

Acute stress experiments: measurement of plasma CORT

Surgery. To permit blood sampling, rats were anesthetized with pentobarbital sodium (50 mg/kg, intaperitoneal [ip]), and a polyvinyl tube was inserted into the jugular vein so that its tip lay in the superior caval vein near the right atrium (Harms and Ojeda 1974). The free end of the catheter was passed subcutaneously to the midscapular region, where it was exteriorized dorsally behind the neck. It was kept patent by flushing it every day with heparinized 0.9% saline (50 U/mL). This implantation was performed at least 3 days prior to the day of the experiment (which included blood sampling).

Measurement of plasma CORT. On the day of the experiment, rats were subjected to 90-min acute restraint stress (9:30 to 11:00) in a small cylindrical restrainer made from steel wire (7 × 22 cm [diameter × length]). Rose essential oil or its vehicle was administered throughout the above period of stress. Blood samples were taken 2 times: at 90 min before the start of and just after the end of the stress. On each occasion, about 0.8 mL of blood was withdrawn, collected into an ice-cooled test tube containing ethylenediaminetetraacetic acid (1 mg/mL blood), and centrifuged at 3000 rpm for 10 min at 4 °C. The plasma was then transferred into a fresh test tube and stored at –80 °C until needed for the measurement of CORT. The plasma concentration of CORT was determined using a commercial CORT radioimmunoassay kit (Corticosterone ^{125}I RIA kit; MP Biomedicals) with a lower detection limit of 7.7 ng/mL.

Acute stress experiments: immunohistochemistry

Tissue collection and immunohistochemistry. Other groups of rats were sacrificed under pentobarbitone anesthesia (100 mg/kg, ip), immediately after the cessation of the same 90-min restraint stress, with or without inhalation of rose essential oil. After perfusion of the animal through the left ventricle with heparinized saline at 37 °C to wash out the blood, the brain was immediately removed, freshly frozen in dry-ice powder, and stored at –80 °C until use. Each brain was then subjected to immunohistochemistry.

The freshly frozen brains were cut at a thickness of 14 μm on a cryostat, then thaw-mounted on silane-coated glass slides (Matsunami Glass Industrial Co., Ltd.). After air drying at room temperature for 30 min, the sections were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 20 min at room temperature. After a rinse with PBS, they were treated with 0.3% H_2O_2 in PBS for 10 min, followed by 3% normal goat serum (NGS; Histofine; Nichirei) and 0.1% Tween 20 in the same PBS. The sections were then incubated overnight at 4 °C with rabbit anti-rat Fos polyclonal antibody (Ab-5; Oncogene Research Products) diluted 1:5000 times in PBS containing 1% NGS and 0.1% Tween 20. The following day, sections were washed with PBS and incubated overnight at 4 °C with the secondary antibody containing biotinylated goat anti-rabbit IgG (1:200; Vector Labs). For diaminobenzidine (DAB) staining, the sections were processed by the conventional avidin-biotin complex (ABC)-DAB method (Elite ABC kit, Vector Labs; and DAB, Histofine; Nichirei). Control staining was conducted without the primary antibody. Bias was avoided by simultaneous processing of sets of samples containing equal representation from each experimental group.

Sections colored with DAB were examined under a microscope (Nikon), and the images were captured by means of a high-resolution CCD video camera system, digitized to 32-bit color images, fed into a Dell computer, and stored as PICT files for further analysis and presentation. The boundaries of the various subdivisions of the PVN were outlined, following the scheme and nomenclature of Swanson and Kuypers (1980) (as later expanded by Swanson and Simmons (1989), Dumont et al. (2000), and Viau and Sawchenko (2002)).

Fos-immunoreactive nuclei in each area were counted as a whole. Then, the resulting number was divided by the surface area and expressed as number per 6.25×10^4 μm^2 area. The planes of sections were standardized as much as possible according to the atlas of Paxinos and Watson (1998). The PVN was counted in 3 sections at the midhypothalamic level, about 1.8 mm posterior to bregma, in a given rat. These counts were then averaged.

Chronic stress experiments: measurement of TEWL and body weight

Rats were restrained as required in a small cylindrical restrainer made of steel wire (7 × 22 cm [diameter × length]) for 8 h (09:30 to 17:30) on each of 14 successive days. Rose essential oil or its vehicle (i.e., triethyl citrate) was administered for 2 h (09:30 to 11:30) on each of these 14 days.

Total body weight was measured just before the start of the restraint stress (i.e., on the first day of the experimental period). In addition, immediately after the end of the stress exposure (i.e., on the 14th day of the experiment), the back skin was shaved under general anesthesia (pentobarbitone sodium, 50 mg/kg, ip) after the rat's body weight had been measured. Thus, changes in total body weight during the experiment could be calculated. The next day (i.e., the 15th day from the start of the experiment), we measured TEWL through the back skin of conscious rats using a cyclone-type moisture-transpiration meter (AS-CT1; Asahibiomed). TEWL was measured 10 times in each animal, with average values being included in our data. During the measurement of TEWL, each rat was handled gently, with its eyes being covered by a hand wearing a glove. In that way, we could measure TEWL from quiet conscious rats. Afterward, animals were sacrificed by CO_2 stunning followed by decapitation.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). Data obtained for the plasma concentration of CORT were analyzed for statistical significance between groups by means of repeated measures analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) test (post hoc test). Student's *t*-test was used when the changes in plasma CORT within a given group were compared before and after the stress. One-way ANOVA, followed by Fisher's PLSD test (post hoc test) (Macintosh, StatView 4.0), was performed for experiments involving 3 groups (immunohistochemistry and TEWL studies). Differences were considered significant at $P < 0.05$.

Human study I

Subjects

The present human study conformed to the principles outlined in the Declaration of Helsinki (*Cardiovascular Research* 1997; 35: 2–4), informed consent was obtained from all subjects, and the protocols were approved by the local Human Subject Ethics Committee in Tottori University Faculty of Medicine. Fourteen healthy women volunteers were recruited from the students in our institution (age 21.0 ± 0.1, body mass index 19.79 ± 0.4 [range 18.0–22.6]). Subjects with olfactory disorders were excluded from the study because an aroma needed to be inhaled. In the present study, hormonal factors, such as those related to the menstrual cycle, were not assessed in the women who acted as subjects.

Rose essential oil

The same rose essential oil (provided by Kanebo Cosmetics) as that used in the rat study was given to the volunteers. Each of them was given an aroma patch set (name tags, aroma test papers, cellophane tape, dropper, and rose essential oil). An aroma patch, consisting of an aroma test paper (rectangular in shape; 75 × 32 mm) impregnated with rose essential oil (1%), was stuck on a name tag, and this was attached to the subject's shirt collar so that she would inhale the essential oil during normal breathing. On each day, the female subjects started to wear the aroma test paper in their home at the time of rising and continued to wear it until they went to bed, except when they were taking a bath. Thus, they did not wear an aroma test paper while they were asleep.

They wore a fresh aroma test paper every day and soaked it with essential oil whenever they felt it had lost its aroma.

Experimental protocols

The female student subjects were due to take the University exams for their promotion in January and February 2010, a situation that we would expect to be a chronic stress to them. Indeed, it has been reported that female students who had taken written and oral exams in the course of the Dental Hygienist Program at the Karolinska Institutet at Huddinge in Sweden showed an increase in salivary cortisol levels during those exams (Johannsen et al. 2010). We measured both the salivary concentration of cortisol and TEWL 21 days before (i.e., before the exams) and 3 days after the start of the exams (i.e., during the exams), and also just after the end of the 13-day exam period (i.e., after the exams). The test group (designated the rose + stress [R + S] group) wore aroma test papers soaked with rose essential oil throughout this experimental period. We asked the control group (designated the vehicle + stress [V + S] group) to wear aroma test papers soaked with vehicle (triethyl citrate) during the same period. The subjects were randomly assigned to the test group or control group.

Measurements of salivary cortisol and TEWL.
Saliva collection tubes (Funakoshi Co. Ltd.) and salivette cotton were used. The cotton was placed in the mouth, and the saliva sample collected about 2 min later. The saliva sample was centrifuged for 15 min at 3000 rpm, placed in microtubes, and stored at –20 °C or below. The saliva concentration of cortisol was measured using a salivary cortisol enzyme immunoassay kit (Catalog No.1-3002; SALIMETRICS).

On each measurement day, TEWL was measured 3 times from the skin of the forearm using a cyclone-type moisture-transpiration meter (AS-CT1; Asahibiomed), and average values were included in our data.

In a separate experiment, the TEWL of each of 20 unstressed female subjects was measured using 2 m (AS-CT1; Asahibiomed and MPA9; Courage + Khazaka Electronic GmbH) to test the accuracy of the meter used in the above experiment (i.e., AS-CT1; Asahibiomed). Figure 1 shows that there was a linear relationship (positive correlation) between the TEWL values measured by the 2 m, with a Pearson product-moment correlation coefficient of 0.99, and $P < 0.05$.

Statistical analysis

All results are expressed as mean ± SEM. Data obtained for the human subjects were analyzed for statistical significance by means of repeated measures ANOVA, followed by Fisher's PLSD test (post hoc test). One-way ANOVA, followed by Fisher's PLSD test (post hoc test) (Macintosh, StatView 4.0), was used when changes in cortisol or TEWL within a given group were compared among before, during, and after the exams. Differences were considered significant at $P < 0.05$.

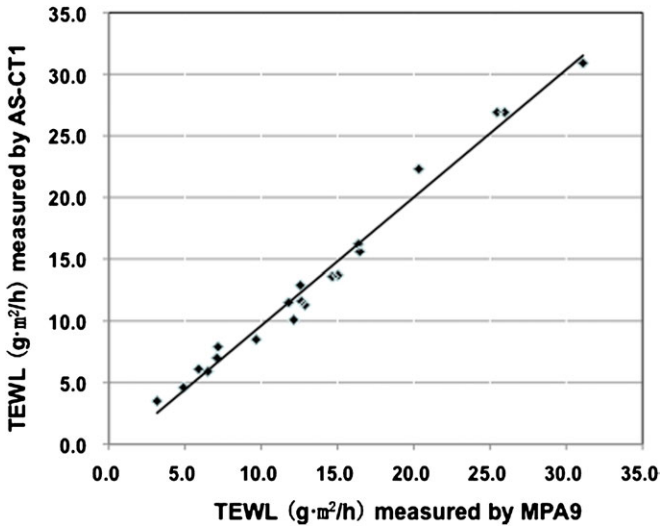

Figure 1 Relationship between the TEWL values measured by 2 m (AS-CT1; Asahibiomed and MPA9; Courage + Khazaka Electronic GmbH). The TEWL value for a given human subject as measured by the AS-CT1 meter is plotted against that obtained using the MPA9 meter (n = 20). There is a linear relationship (positive correlation) between the values measured by the 2 m (Pearson product-moment correlation coefficient, 0.99; $P < 0.05$).

Human study II

Subjects

To examine whether a placebo effect might be involved in the observed effects (in Human study I) of rose essential oil on stress-induced responses in human subjects, another 13 healthy women volunteers were recruited from the students in our institution (age 21.23 ± 1.01, body mass index 20.49 ± 1.86 [range 18.31–22.83]).

Odorants

Each subject was exposed to vehicle (triethyl citrate) and 2 essential oils (1%): namely, rose essential oil (the same as that used in Human study I) and jasmine essential oil (as a control odorant). The jasmine essential oil, derived from *Jasminum sambac*, was purchased from Biolandes. The way in which the subjects were exposed to the odorants was the same as in Human study I, except that they inhaled a given odorant during an acute stress test lasting about 20 min (see Experimental protocols). In each subject, the 3 inhalants were presented in a randomized order, one of them on the first, another on the fourth, and the last on the seventh day of her participation in the study, with the other days being "blank" days. We explained to the subjects that one or both of the oils might reduce their response to the concomitant acute stress.

Experimental protocols

Acute mental stress was induced by a classic version of the Stroop color-word test (Stroop 1935). Briefly, the participants

were asked to name the ink color used to print each of a series of words, denoting a different color (e.g., the correct response to the word "blue," when printed in red ink color, is "red"). The subject's task is to look at each sheet, reading the words and naming the ink colors as quickly as possible. For each subject, the session lasted for about 20 min.

Saliva samples were taken just before the start of and immediately after the end of this 20-min mental stress. The saliva concentration of cortisol was measured using a salivary cortisol enzyme immunoassay kit (Catalog No.1-3002; SALIMETRICS).

Statistical analysis

Results are expressed as mean ± SEM. Data were analyzed for statistical significance by means of repeated measures ANOVA, followed by Fisher's PLSD test (post hoc test). Student's *t*-test was used when the changes in saliva cortisol within a given group were compared before and after the stress. One-way ANOVA, followed by Fisher's PLSD test, was performed to compare the saliva level of cortisol before the stress between the groups.

Results

Rat study

Acute stress experiments: measurement of plasma CORT

As shown in Figure 2, a significant increase in the plasma concentration of CORT was observed just after the end of 90-min restraint stress, as compared with that at 90 min before the start of the stress exposure, in the R + S group and in the V + S group. When the 2 groups were compared, the R + S group showed a significantly smaller CORT response than the V + S group.

Acute stress experiments: immunohistochemistry

Figure 3 shows effects of 90-min restraint stress on neuronal activities in the PVN, with immunoreactivity for Fos protein being used as the index. It is well known 1) that 3

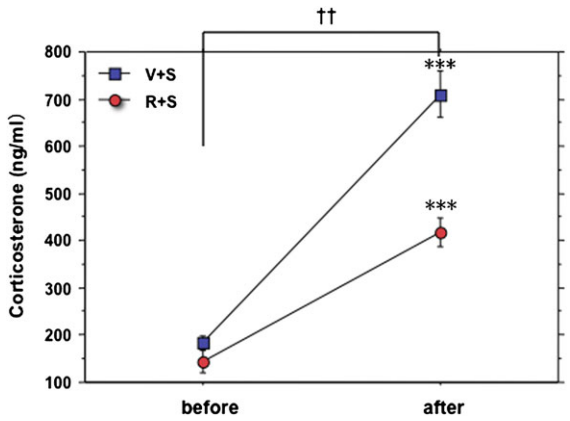

Figure 2 Effects of rose essential oil on acute restraint stress-induced change in plasma concentration of corticosterone (CORT) in rats. Mean values (±SEM; $n = 5$ each) obtained for plasma CORT before and after acute stress in rats, in the absence (V + S group) or presence (R + S group) of rose essential oil inhalation. $***P < 0.001$ versus before. Bracket indicates significant difference between the groups of rats during the course of the experiment ($††P < 0.01$). This figure appears in color in the online version of *Chemical Senses*.

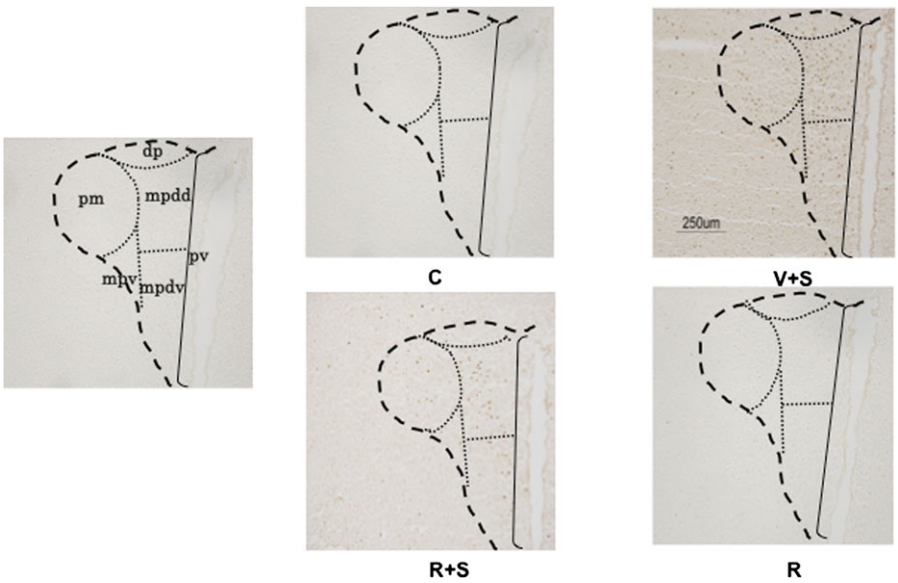

Figure 3 Fos expression in rat hypothalamic PVN, effects of rose essential oil on changes induced by acute restraint stress. Fos-immunoreactive neurons in the hypothalamic PVN in acutely stressed rats. Results are shown for the absence (V + S group) or presence (R + S group) of inhalation of rose essential oil. Effects of rose essential oil are also shown for nonstressed animals (R group), as are the data for rats given no treatment at all (C group). The outlines of the various subdivisions of the PVN are superimposed on each panel. This figure appears in color in the online version of *Chemical Senses*.

subdivisions of the parvicellular PVN—namely, the dorsal and ventral bodies of the medial parvicellular dorsal region (mpdd and mpdv, respectively) and the periventricular (pv) region—give rise to hypophysiotropic projections and 2) that 2 other subdivisions, the dorsal parvicellular (dp) and medial parvicellular ventral (mpv) parts, are preautonomic regions. In addition, the posterior magnocellular (pm) PVN is packed with vasopressinergic and oxytocinergic neurons projecting to the posterior pituitary (the various subdivisions are labeled in the left panel in Figure 3). As compared with the C group, all of these regions displayed increases in Fos expression after the stress (V + S group), and these effects were inhibited by rose essential oil inhalation (R + S group; Figure 3). The rose essential oil itself had no effect on Fos expression within the PVN (R group).

Figure 4 shows the results of our quantitative analysis of Fos immunohistochemistry in PVN. In all subdivisions of PVN, Fos-immunoreactive responses were significantly greater in the V + S group than in the C group. Furthermore, in each subdivision, the number of Fos-positive cells was significantly smaller in the R + S group than in the V + S group. However, this inhibition was partial because the number of Fos-positive cells in the R + S group was, or tended to be, greater than that in the C group. The R group showed no significant increase in Fos-positive cells (vs. the C group) in any PVN region.

Chronic stress experiments: measurement of TEWL

As depicted in Figure 5, imposition of chronic stress (8 h per day for 14 days) resulted in a significant increase in TEWL (V + S group vs. C group). This effect was completely suppressed by rose essential oil inhalation (R + S group), no significant difference in TEWL values being observed between the R + S and C groups.

Table 1 shows that body weight was decreased after 14-days' chronic stress in the V + S group (vs. the C group). Inhalation of rose essential oil had no significant effect on such weight loss (R + S group vs. V + S group).

Human study I

Measurement of saliva cortisol

The salivary levels of cortisol before, during, and after the exams are shown in Figure 6. The control group, who did not inhale the rose essential oil, showed a significant increase in the salivary concentration of cortisol both during and after the exams, as compared with the level before the exams. However, no significant changes in salivary cortisol were observed in the group subjected to rose essential oil inhalation. Furthermore, as indicated in Figure 6, the overall cortisol levels in the R + S group were significantly smaller than those in the control V + S group.

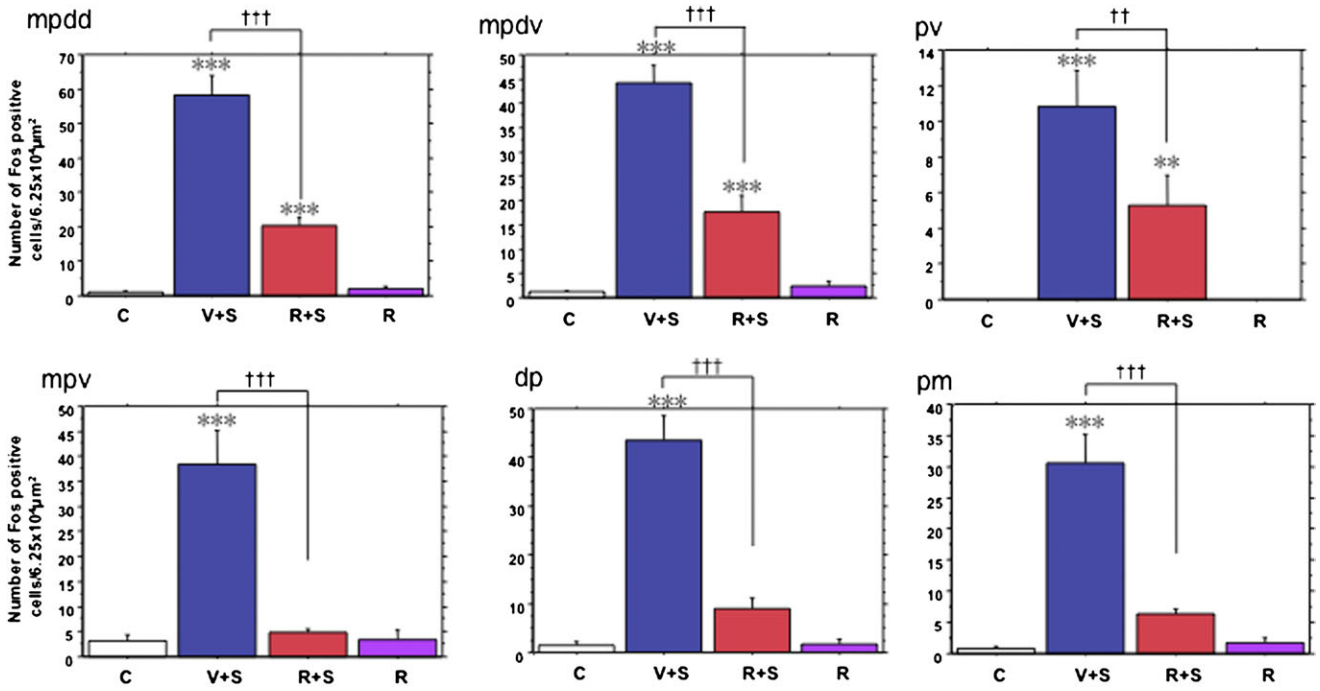

Figure 4 Quantitative analysis of Fos-positive neurons in the PVN of rats exposed to acute restraint stress with or without inhalation of rose essential oil. Mean values (±SEM) obtained for the number of Fos-positive neurons in the various subdivisions of PVN in acutely stressed rats. Data are shown for the absence (V + S group; $n = 6$) or presence (R + S group; $n = 6$) of rose essential oil inhalation. Data are also shown for nonstressed animals exposed to rose essential oil (R group; $n = 5$) and for animals given no treatment at all (C group; $n = 8$). $^{**}P < 0.01$ versus C group and $^{***}P < 0.001$ versus C group. Significance of differences between groups V + S and R + S is indicated above bracket ($\dagger\dagger P < 0.01$ and $\dagger\dagger\dagger P < 0.001$). This figure appears in color in the online version of *Chemical Senses*.

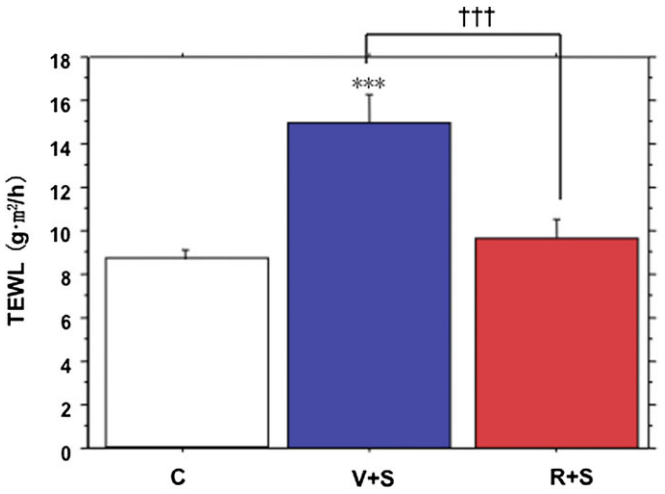

Figure 5 Effects of rose essential oil on chronic restraint stress-induced change in TEWL in rats. Mean values (±SEM) obtained for TEWL in chronically stressed rats, in the absence (V + S group; $n = 6$) or presence (R + S group; $n = 6$) of rose essential oil inhalation. Also shown are the data for rats given no treatment at all (C group; $n = 9$). $^{***}P < 0.001$ versus C group. Significance of difference between groups V + S and R + S is indicated above bracket ($\dagger\dagger\dagger P < 0.001$). This figure appears in color in the online version of *Chemical Senses*.

Table 1 Effects of rose essential oil on chronic restraint stress-induced change in body weight in rats

	Change in body weight (g)
C	29.3 ± 2.41
V + S	−40.5 ± 4.65***
R + S	−47.6 ± 2.01***

Mean changes (±SEM; $n = 6$ each) obtained for body weight in chronically stressed rats, in the absence (V + S group; $n = 6$) or presence (R + S group; $n = 6$) of rose essential oil inhalation. Also shown are the data for rats given no treatment at all (C group; $n = 9$).
$^{***}P < 0.001$ versus C group.

Measurement of TEWL

The V + S control group displayed a significant increase in TEWL during the exams, whereas there were no changes at all in TEWL in the R + S group (Figure 7). TEWL values obtained during and after the exams for the test group, who inhaled the rose essential oil, showed evidence of significant suppression versus those obtained for the control group.

Human study II

Measurement of saliva cortisol

In the vehicle-exposure (control) sessions, 20-min acute mental stress (Stroop color-word test) evoked a significant increase in salivary concentration of cortisol (after vs. before stress; Figure 8). This cortisol response was significantly smaller in the rose oil–exposure sessions, whereas

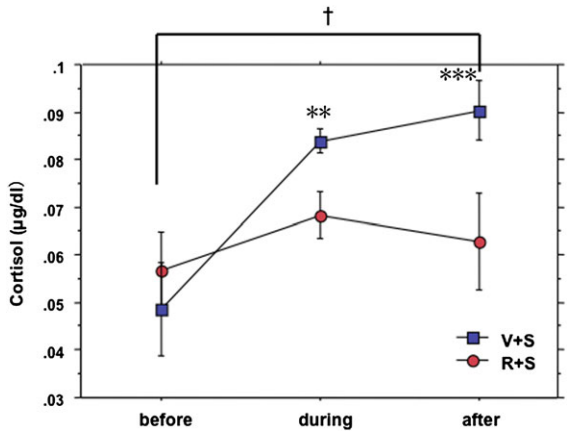

Figure 6 Effects of rose essential oil on chronic stress-induced changes in salivary concentration of cortisol in humans. Mean values (±SEM; $n = 7$ each) obtained for changes in salivary cortisol concentration in chronically stressed humans, in the absence (V + S group) or presence (R + S group) of rose essential oil inhalation. $^{**}P < 0.01$ versus before and $^{***}P < 0.001$ versus before. Bracket indicates significant difference between groups of subjects during the course of the experiment ($\dagger P < 0.05$). This figure appears in color in the online version of *Chemical Senses*.

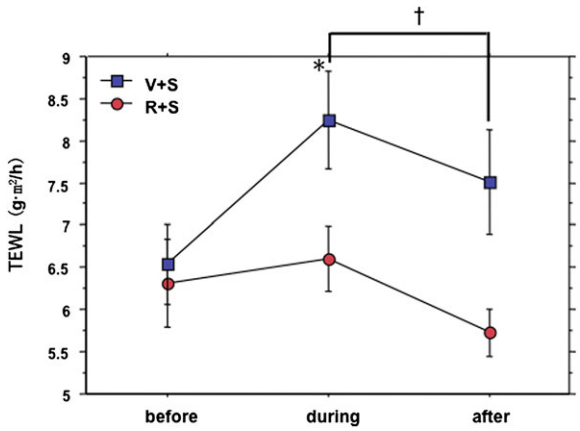

Figure 7 Effects of rose essential oil on chronic stress-induced changes in TEWL in humans. Mean values (±SEM; $n = 7$ each) obtained for TEWL in chronically stressed humans, in the absence (V + S group) or presence (R + S group) of rose essential oil inhalation. $^{*}P < 0.05$ versus before. A significant difference between the groups of subjects was established for the period indicated by the bracket ($\dagger P < 0.05$). This figure appears in color in the online version of *Chemical Senses*.

no significant difference in this response was observed between the control-exposure and the jasmine oil–exposure sessions (analysis by repeated measures ANOVA). However, it should be noted that in the jasmine oil–exposure sessions, the apparent increase in the salivary concentration of cortisol just after the stress was nonsignificant (vs. before stress). Subjects who inhaled rose essential oil during the stress exhibited almost no change in salivary cortisol after the stress. Before the stress, there were no significant differences in the salivary level of cortisol among the 3 groups (V + S, R + S, and J + S, see Figure 8) (analysis by one-way ANOVA).

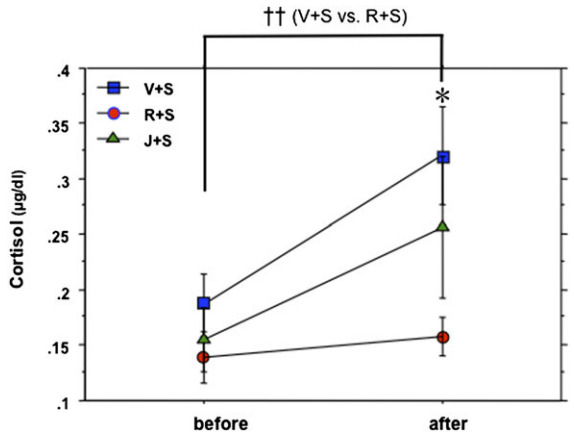

Figure 8 Effects of rose essential oil or jasmine essential oil on acute stress-induced changes in salivary concentration of cortisol in humans. Mean values (±SEM; n = 13 each) obtained for salivary cortisol concentration in acutely stressed humans, in the absence (V + S) or presence of essential oil inhalation (either rose essential oil [R + S] or jasmine essential oil [J + S]). *P < 0.05 versus before. A significant difference between the vehicle and rose oil groups (V + S vs. R + S) was established for the period indicated by the bracket (††P < 0.01). This figure appears in color in the online version of *Chemical Senses*.

Discussion

The present study examined the effects of rose essential oil on 2 major stress-induced effects, activation of the HPA axis and skin-barrier disruption, in rats and humans. In rats, acute restraint stress evoked an increase in plasma CORT that was significantly inhibited by inhalation of rose essential oil. Furthermore, when the PVN was divided into 6 subdivisions, the acute stress-induced increases in the number of c-Fos-positive cells were significantly reduced in all subdivisions by inhalation of that oil. Those PVN subdivisions regulate either the activation of the HPA axis (the mpdv, mpdd, and pv) or the sympathetic nervous system (the dp and mpv) (Herman et al. 2002). Furthermore, vasopressinergic and oxytocinergic neurons projecting to the posterior pituitary originate from the pm PVN. It should be noted that Fos expression was "partially" inhibited in the mpdd, mpdv, and pv of the PVN but "completely" suppressed in the mpv, dp, and pm of the PVN. This result suggests that upon inhalation of rose essential oil, although stress-induced activation of the HPA axis is inhibited, the inhibition is partial, although such activation of the sympathetic nervous system and/or of the vasopressinergic and oxytocinergic neurons projecting to the posterior pituitary may be completely suppressed. Possibly, the sensitivity to and/or the mechanisms responsible for such inhibition may differ between those systems. Collectively, the above results suggest that in rats, inhaling rose essential oil results in some degree of inhibition of the activity of neurons within the PVN and that this in turn leads to inhibitions of the activation of the HPA axis (e.g., increase in plasma CORT), sympathetic nervous system, and posterior pituitary system induced by acute stress.

In chronic stress experiments, we measured TEWL as an indicator of skin-barrier disruption. The results showed that in both rats and humans, the chronic stress-induced elevation of TEWL was significantly inhibited by inhalation of rose essential oil. It has been reported that in mice, skin-barrier disruption (as evidenced by an increase in TEWL) occurs after chronic exposure to stress (Aioi et al. 2001). Furthermore, we recently showed that in rats, chronic stress-induced disruption of the skin barrier can be reduced or prevented by the inhalation of green odor (possibly at least in part through an inhibitory effect on the stress-induced activation of the HPA axis) (Fukada et al. 2007). Therefore, the inhibitory effects of rose essential oil on the HPA axis described above might also underlie the observed prevention of chronic stress-induced skin disruption. Indeed, in this study, not only in rats but also in humans, stress-induced increases in the plasma and salivary concentrations of glucocorticoids were inhibited by inhalation of rose essential oil, even though the rats and humans were exposed to different periods of stress (acute or chronic stress, respectively). In future experiments, the effect of rose essential oil on the chronic stress-induced change in plasma CORT needs to be examined in rats too.

At this juncture, we should mention that we did not show an exact relationship between the effect of rose essential oil on HPA and the observed protection against skin-barrier disruption. However, in our previous paper, on rats, we presented results suggesting 1) that an increased secretion of glucocorticoid during our "stress experiment" might have been an important factor in the observed impairment of the skin barrier and 2) that this increased secretion was attenuated by inhalation of green odor, leading to at least a partial prevention of the skin-barrier disruption induced by such chronic stress (Fukada et al. 2007). On that basis, it is possible that inhibitory effects of rose essential oil on the HPA axis underlie the observed prevention of chronic stress-induced skin-barrier disruption. To test that idea, we need to determine the exact correlation between the reduction in HPA activity and the skin-barrier protection induced by rose essential oil.

Collectively, this report and our previous ones (Fukada et al. 2007; Ito et al. 2009) indicate that rose essential oil and green odor each inhibit the observed stress-induced effects on the HPA axis and skin-barrier function. However, in the present study on rats, there was no difference in the chronic restraint stress-induced decrease in body weight between the rose essential oil-exposed and control groups. In contrast, we previously showed that green odor inhibited this body weight response (Fukada et al. 2007). This discrepancy may indicate some difference(s) in the mechanisms by which odorants induce effects on such stress-induced responses. Recently, we reported that inhalation of green odor by stressed rat dams prevented both depression-like behavior and an increased responsiveness of the HPA axis to stress being exhibited by the dam's offspring (Fujita et al. 2010). However, the effects of rose essential oil on such prenatal stress-induced behavioral and hormonal alterations have

yet to be examined. In order to explore the specificity of the present effects of rose essential oil, its effects on the above prenatal stress-induced changes could be examined.

The mechanism underlying the suppressive effects of rose essential oil on the HPA axis and TEWL is unknown, but 2 possibilities occur to us. One is that the perfume component of rose essential oil is absorbed into the blood via the membrane of the nose or lung and that this absorbed component triggers a certain reaction in the brain and/or skin, with consequent reductions of stress-induced HPA activation and skin-barrier disruption. The other possibility is that upon inhalation, the oil activates the olfactory nervous pathway and that this leads to inhibition of the activities of stress-related forebrain regions, such as PVN, an effect that might possibly be responsible for the inhibition of skin-barrier disruption, as reported in the case of green odor by Fukada et al. (2007). Another interesting point is that the scents of rose oil and green odor are quite different, yet their effects, or at least those observed so far, are similar. We cannot attempt a clear explanation for this at the present time. However, Denda et al. (2000) reported that 4 different odorants, each with a sedative effect, prevented the delay in skin-barrier recovery induced by stress after acute barrier disruption. Hence, it is likely that the ''sedative'' effects of odorants, after absorption and/or stimulation of the olfactory pathway, are important in inducing inhibition of stress-induced responses.

Two components of our rose essential oil, eugenol and linalool, have been reported to be able to inhibit HPA activity (Höferl et al. 2006; Garabadu et al. 2011). Furthermore, phenylethyl alcohol reportedly prevented the delay in skin-barrier recovery induced by stress after acute barrier disruption (Denda et al. 2000). To judge from those results, eugenol, linalool, and phenylethyl alcohol might have been important in inducing the present suppression of the stress-induced activation of the HPA axis and skin-barrier disruption. However, other components may play such roles too. This will need to be investigated in future experiments.

Usually, it is necessary to take into account the possibility of a placebo effect in human studies. In our Human study II, subjects inhaled jasmine essential oil as a control odor, with the subjects having been told that this oil, and/or the rose oil, might reduce their stress-induced response. The present results showed that the overall cortisol response induced by acute mental stress was significantly attenuated by inhalation of rose essential oil, whereas the jasmine oil did not have a significant effect, suggesting that the inhibition of the stress-induced cortisol response by rose essential oil was not simply a placebo effect. However, because the increase in salivary cortisol level induced by stress in the jasmine oil–exposed sessions did not reach statistical significance, we cannot completely exclude the possibility of a placebo effect. Conceivably, the reduction, by inhalation of the rose essential oil, of the cortisol response might involve a major direct effect and some degree of placebo effect in human subjects. The idea that inhalation of rose essential oil may have significant direct (nonplacebo) effects is supported by the finding that in our hands, there were no differences in State-Trait Anxiety Inventory scores or in Profile of Mood States scores between the test (rose oil) and control (vehicle) subjects (data not shown). In other words, placebo effects were not observed in those tests, which are more subjective than the hormonal and TEWL studies. Furthermore, in rats, exposure to rose essential oil led to inhibition of stress-induced HPA axis and TEWL responses, and similar effects of the oil might be anticipated in humans.

The present results represent the first evidence that both in rats and in humans, inhaling rose essential oil has an inhibitory effect on chronic stress-induced skin-barrier disruption, possibly through an inhibition of the activation of the HPA axis induced by the stress. The major component of the skin barrier is located in the outermost layer of the skin, the stratum corneum, which consists of corneocytes surrounded by lipid regions (Elias 2005). These intercorneocyte lipids play a very important role in skin-barrier function (Rawlings and Harding 2004) and are reportedly reduced in chronically stressed animals (Aioi et al. 2001; Choi et al. 2005). We recently reported that inhalation of green odor prevented the decrease in intercorneocyte lipids seen in chronic stress-exposed rats (Fukada et al. 2007). A similar histological examination will need to be performed to determine whether the chronic stress-induced decrease in intercorneocyte lipids actually is limited or prevented by rose essential oil as well. We did not monitor hormonal factors, such as those related to the menstrual cycle, in our female human subjects. It would be interesting to examine if or how the menstrual cycle affects the rose essential oil–induced effects on activation of HPA axis and skin-barrier disruption induced by stress.

In conclusion, in humans as well as in rats, inhalation of rose essential oil holds promise as a strategy for the prevention or relief of at least some stress-induced effects, such as undue activation of the HPA axis and disruption of the skin barrier.

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