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Subjective unpleasantness of malodors induces a stress response

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ABSTRACT

Unpleasant odors impair our mood and may affect physical health, even when the odorants are not toxic. A possible cause for such negative effects is stress induced by odors; however, whether the unpleasantness itself elicited stress or not has not been clear. Thus, we examined whether unpleasantness of odors induced the stress responses of emotion, the hypothalamic-pituitary-adrenal (HPA) axis, and the sympathetic nervous system (SNS). Six experiments were conducted, where salivary cortisol or salivary alpha amylase (sAA), markers for activities of the HPA and the SNS, respectively, were measured, along with subjective ratings of odors and emotion. First, the responses to three malodors listed in the Offensive Odor Control Law in Japan were examined. While these odors were rated as unpleasant, and exposure to them increased anxiety, no response of the HPA was observed (experiment 1, n = 69). In contrast, an increase of the SNS activity was observed after exposure to two of the three malodors, while the SNS did not respond to pleasant odors (experiments 2–4, n = 35, 34 and 30). To examine the effect of unpleasantness further, the SNS response was examined while subjective unpleasantness of odors was manipulated by adding negative verbal information (experiment 5, n = 92), or by mixing in a pleasant odor (experiment 6, n = 35). The SNS responses upon inhalation of the same odorous substances were found to be dependent on whether they were perceived as unpleasant. Finally, a correlation analysis on the pooled data from experiments 2–6 showed that the odor-elicited SNS activity and anxiety were strongly correlated with perceived unpleasantness of odors. These results suggest that subjective unpleasantness of odors per se can induce the stress response of emotion and the SNS.

1. Introduction

Unpleasant odors impair our mood and sense of wellbeing, and may even cause physical symptoms. Malodors emitted from paper mills were strongly associated with eye, respiratory, and neuropsychological symptoms (Haahtela et al., 1992). Neighbors of industrial hog operations suffer from odors, and were found to have decreased levels of immunoglobulin A and increased blood pressure (Avery et al., 2004; Wing et al., 2013). Some odorous molecules are toxic to humans and could affect health (Schiffman and Williams, 2005). However, negative effects may occur even when toxicity or irritation is unlikely to be present. For example, common daily odors in office environments such as body odor, food odor, or perfume were found associated with general symptoms such as headache or unusual tiredness in those individuals who perceived the odors as unpleasant (Azuma et al., 2015).

The mechanism responsible for such negative effects caused by nontoxic odors is not well understood. One hypothesis is that it is not the odorous substances per se, but the unpleasantness of odors that induces stress, and this stress leads to further adverse effects (Schiffman and Williams, 2005; Smeets and Dalton, 2005). Individuals exposed to environmental malodors are reported to show increased levels of subjectively reported stress and negative mood (Avery et al., 2004; Horton et al., 2009; Wing et al., 2013). One of these studies further showed association between subjective stress and a physical symptom, in this case, blood pressure (Wing et al., 2013). However, studies that reported an association between malodor and stress were field surveys in which various factors other than odors, such as dust or toxic substances, were not controlled for. In addition, the conclusions relied on subjective reports to evaluate stress. Therefore, it is unclear whether the unpleasantness of the odors itself elicits stress responses.

A stressful situation can increase subjective feelings of unease, tension, and stress, which can be referred to as anxiety (Rossi and Pourtois, 2012). It can also trigger a cascade of physiological changes mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the

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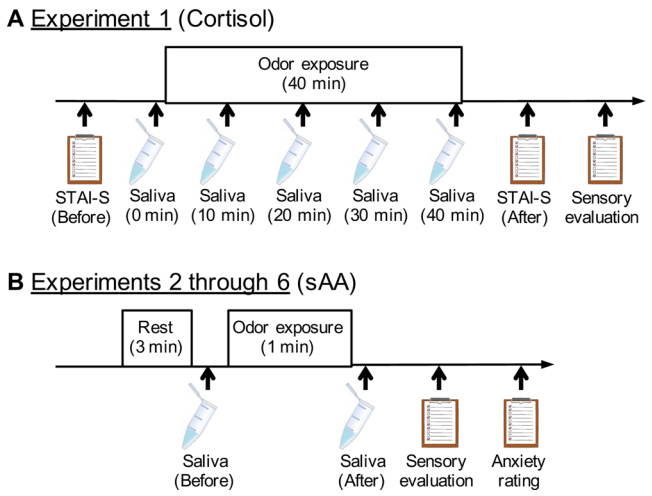

Fig. 1. Experimental paradigms.
Timeline of an experimental session for the cortisol experiment (A) and the salivary alpha amylase (sAA) experiments (B). In the cortisol experiment, each participant took part in two sessions, one for odor and the other for control, conducted on separate days. For sAA, sessions were repeated for the number of samples for each experiment, and all of the sessions for each experiment were conducted on the same day. STAI-S, State Trait Anxiety Inventory-State.

sympathetic nervous system (SNS) (Chrousos, 2009). When the HPA axis is activated, glucocorticoids, mainly cortisol in humans, are released from the adrenal glands, and glucocorticoids change the physiological state (Smith and Vale, 2006). The SNS also changes vital functions rapidly by sending signals to target organs (Kyrou and Tsigos, 2009). While these stress responses are primarily adaptive, prolonged or over activation of the stress systems affects physical and mental health (Everly and Lating, 2013).

There are studies that examined the activation of the SNS upon exposure to odors in laboratory-based research paradigms where, unlike field surveys, factors other than odors, such as dust are controlled. Although some studies did not show a relationship between the unpleasantness of odors and SNS activities in any of the markers that they measured (Bensafi et al., 2002c; Miltner et al., 1994; Moller and Dijksterhuis, 2003), the majority of these studies did find a change in SNS activity, for at least one of the markers they measured (Alaoui-Ismaili et al., 1997; Bensafi et al., 2002a, b; Croy et al., 2013; Delplanque et al., 2008, 2009; Glass et al., 2014; He et al., 2014, 2016; Pichon et al., 2015). However, as the focus of these studies was not the stress response, they did not examine the stress-related emotional state, and its association with the SNS responses. In addition, previous reports on odor and the HPA focused on stress-relieving effects (e.g., Fukada et al., 2012; Nishitani et al., 2009), and the effect of unpleasant odors on the HPA axis in humans, to the best of our knowledge, has not been examined.

Regarding whether subjective unpleasantness of odors itself can elicit stress response or not, it is generally difficult to distinguish the effect of odorous substances, and the unpleasantness the substances raise. One approach is to manipulate the perceived unpleasantness of the same odorous substances by changing the context of their presentation. The pleasantness of odors can substantially change depending on the context in which the odor was inhaled. For example, odors presented with negative information, such as vomit, tend to be perceived as more unpleasant than those presented with neutral or pleasant information (Djordjevic et al., 2008; Herz, 2003; Herz and von Clef, 2001; Stevenson, 2011). Another prominent phenomenon is the odor masking effect, where an addition of odors suppresses the unpleasantness of the original odor (Thomas-Danguin et al., 2014). Such contextual effects allow us to examine the stress response to the inhalation of same amount of malodourous substances perceived with different levels of unpleasantness.

To our knowledge, so far, there are only two studies that have examined the effect of context on physiological reactions to odors, and neither were focused on stress. In one study, an odor was presented with a description of eliciting asthma symptoms (Jaen and Dalton, 2014). While self-reported symptoms increased, modulation of SNS activities was not found. As their focus was not on malodors, they did not report pleasantness of odors. Another study examined the context effect on both pleasantness and SNS responses. Significant effects of negative verbal information were found on odor pleasantness and one of the SNS measures used (Djordjevic et al., 2008). To our knowledge, there is no study that examined the odor masking effect on a stress response to odors. Since the topic is understudied, it is worth examining how the unpleasantness modulated by context influences stress responses.

Therefore, we examined whether exposure to malodors induced stress responses associated with emotion, the HPA, and the SNS, and if so, whether they were influenced by the perceived unpleasantness of odors. Salivary cortisol and salivary alpha amylase (sAA) (Keremi et al., 2017; Nater and Rohleder, 2009) were used as markers of the HPA axis and SNS, respectively. Subjective ratings of anxiety and odor perception were also measured to examine the association between subjective pleasantness of odors and the stress responses. In this way, we aimed to elucidate the effects of unpleasantness of malodors on physical and emotional stress responses.

2. Overall study design

Since the temporal dynamics of stress responses are different for salivary cortisol and sAA (Engert et al., 2011), we examined them separately using an experimental design suitable for each parameter (Fig. 1). Six experiments were conducted. In experiment 1, salivary cortisol was measured upon exposure to malodors to examine the response of the HPA axis. In experiments 2 through 4, sAA was measured upon exposure to the same malodors used in experiment 1, as well as some pleasant odors to examine the response of the SNS. In experiments 5 and 6, the effects of odor presentation contexts on the SNS responses to odors were examined using verbal information (experiment 5) and the masking effect (experiment 6). In all of the experiments, subjective ratings of odors and anxiety were obtained together with the physiological measure.

The study was approved by the ethics committee of the University of Tokyo and was performed in accordance with the Declaration of Helsinki. The experiments were conducted after obtaining written informed consents from the participants.

2.1. Experiment 1: effects of malodors on the secretion of salivary cortisol

To examine the effects of malodors on the HPA axis, we presented malodors and measured the odor perceptions, anxiety, and the secretion of cortisol. To verify that the HPA response to stress was detectable using our experimental setting, we also conducted a control experiment, in which changes of cortisol in response to the cold pressor test (CPT; Schwabe et al., 2008), a task known to activate HPA, were examined.

2.1.1. Methods

Three malodors (IVA, DMDS and n-VAL) selected from among odor substances specified as offensive in the Offensive Odor Control Law of Japan (Ministry of the Environment Government of Japan, 1995) were used as odor samples (Supplementary Table 1). Empty bottles were used in the odorless control condition.

Response to each malodor was examined using an independent

participant group (IVA, n = 19; DMDS, n = 19; n-VAL, n = 22; Supplementary Table 2). Each group participated in two sessions, one for odor, and the other for control, conducted on separate days. During the odor exposure, saliva was collected by having each participant drool into a polypropylene tube (Fig. 1A). Stress-related emotional state was assessed using the State-Trait Anxiety Inventory-State (STAI-S). Sensory evaluation was conducted to obtain pleasantness, intensity and familiarity ratings of the odor samples. In the positive control experiment, a separate group of participants (n = 9; Supplementary Table 2) underwent CPT, instead of exposure to odors.

Cortisol levels of the saliva samples were assessed using the Expand Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, LLC.). Statistical tests were performed to compare odor and control conditions within each participant group (alpha = .05, 2-tailed). The Bonferroni method was used for multiple testing corrections. Multiplicity adjusted p-values were reported unless stated otherwise. If p-values became larger than 1, they were reported as $P = 1$.

Details are provided in the Supplementary Materials and Methods.

2.1.2. Results

Odor ratings showed that all the malodors were perceived as unpleasant, strong and familiar (Figs. 2A, S1A, S2A). Among malodors, IVA and DMDS were rated as especially unpleasant (mean rating values corresponded to “unpleasant” to “very unpleasant”), while the mean rating value for n-VAL corresponded to “somewhat unpleasant”.

Anxiety, measured using STAI-S questionnaire, was significantly increased by exposure to IVA and DMDS (Fig. 2B). For the IVA and DMDS groups, two-way interaction between session (before / after) and condition (malodor / control) was significant (IVA, $F_{1,54} = 12.10$, $P \leq .01$; DMDS, $F_{1,54} = 14.96$, $P \leq .01$). When the simple main effects were examined, STAI-S scores were significantly higher in the “after” sessions compared to the “before” sessions in the malodor conditions (IVA, $t(18) = 4.28$, $P \leq .01$; DMDS, $t(18) = 3.15$, $P \leq .01$). STAI-S scores were also significantly higher in the malodor than in the control conditions in the after session questionnaire (IVA, $t(18) = 2.81$, $P \leq .01$; DMDS, $t(18) = 4.11$, $P \leq .01$). For n-VAL, there was no significant interaction between session and condition for STAI-S scores (n-VAL; $F_{1,63} = 3.20$, $P = .08$) (Fig. 2B).

As seen in Fig. 2C, the time course changes in salivary cortisol levels in response to exposure to odor were similar to control conditions for all of the groups. In order to evaluate the influence of malodor on the changes in salivary cortisol level, we used linear multilevel models. Participant identity was entered as a random factor, and time (0–40 min), conditions (malodor/control) and their interaction as fixed effect factors. Significant interactions of time and condition were not observed in any of the groups (IVA, $F_{4,162} = .19$, $P = .94$; DMDS, $F_{4,162} = .15$, $P = .97$; n-VAL, $F_{4,189} = .31$, $P = .87$).

To confirm that the lack of effects of malodors on salivary cortisol was not due to methodological limitations, we examined cortisol response to the CPT. Significant interactions of time (0–40 min) and condition (CPT/control) were observed ($F_{5,88} = 3.69$, $P \leq .01$) (Fig. 2D). Follow-up *post hoc* tests on the simple main effects (p-values not corrected for multiplicity) showed that the concentration of cortisol was significantly higher at 20 min after the onset of the CPT than baseline ($t(8) = 2.53$, $P \leq .05$). The secretion of cortisol was also significantly higher in the CPT than in the control task at 10–40 minutes after the onset of task (10 min, $t(8) = 2.33$, $P \leq .05$; 20 min, $t(8) = 2.76$, $P \leq .05$; 30 min, $t(8) = 2.69$, $P \leq .05$; 40 min, $t(8) = 2.59$, $P \leq .05$).

These results suggest that exposure to malodor does not influence secretion of salivary cortisol even when odors are highly unpleasant (i.e., IVA and DMDS) and elicit anxiety. As we could successfully detect significant HPA response to the CPT, the null finding with malodor was unlikely to be due to a simple technical error.

2.2. Experiment 2: effects of malodors on the secretion of sAA (various odors)

To examine whether malodor affects stress responses related to the SNS, we measured the secretion of sAA along with odor and anxiety ratings.

2.2.1. Methods

Five odors were used: the same malodors as in experiment 1 (IVA, DMDS, n-VAL), and two pleasant odors (MUS, VAN; Supplementary Table 1). Empty bottles were used in the odorless control condition.

Since changes in sAA secretions occur much faster than for salivary cortisol (Engert et al., 2011), an experimental session shorter than that of experiment 1 was used for each odor (Fig. 1B). Sessions were repeated for a number of samples (5 odors and 1 control), and a single group of participants underwent all the sessions (n = 35; Supplementary Table 3). Subjective anxiety was assessed using a short questionnaire that consisted of six questions selected from STAI-S.

sAA activity of saliva samples was measured using the Salivary α-Amylase Kinetic Enzyme Assay Kit (Salimetrics, LLC.). Since distributions of the sAA values were not normal, non-parametric tests were used. Otherwise, the methods were the same as in experiment 1.

Details are provided in the Supplementary Materials and Methods.

2.2.2. Results

Participants’ ratings of odor samples confirmed that all the malodor samples were unpleasant, with pleasant samples rated as pleasant (Fig. 3A, left). Perceived intensities were generally higher for malodors, while familiarity of odors was almost the same across samples except for VAN (Figs. S1B, S2B).

Anxiety was significantly increased after exposures to malodors when compared to control (IVA, $t(34) = 7.41$, $P \leq .01$; DMDS, $t(34) = 6.33$, $P \leq .01$; n-VAL, $t(33) = 3.68$, $P \leq .01$) while they were not significantly different after exposure to pleasant odors (MUS, $t(34) = -1.93$, $P = .30$; VAN, $t(33) = -2.53$, $P = .08$) (Fig. 3A, middle).

Changes in sAA secretion upon odor exposure are shown in Fig. 3A, right. The highest median sAA value was observed in the IVA condition. When the sAA values for each odor condition were compared to that of control, sAA values were significantly higher under the IVA and DMDS conditions (IVA, $Z = 2.85$, $P \leq .01$; DMDS, $Z = 3.41$, $P \leq .01$). The sAA values under the other odor conditions were not significantly different from that of control (n-VAL, $Z = .13$, $P = 1$; MUS, $Z = -.46$, $P = 1$; VAN, $Z = -.90$, $P = 1$).

Considering that significant sAA increases were observed for the top two most unpleasant odors tested, the results indicate that exposure to highly unpleasant odors increases secretion of sAA.

2.3. Experiment 3: effects of malodors on the secretion of sAA (0.01–10% DMDS)

To examine the reproducibility of experiment 2, and to further examine the effect of unpleasantness of odors on the SNS, we measured sAA secretions to a malodor presented at different concentrations.

2.3.1. Methods

We presented 0.01%, 0.1%, 1%, and 10% DMDS (Supplementary Table 1) to a separate group of participants (n = 34; Supplementary Table 3). Otherwise, the methods were the same as in experiment 2.

Details are provided in the Supplementary Materials and Methods.

2.3.2. Results

DMDS presented at the same concentration used in experiment 2 (10% DMDS) was rated as similarly unpleasant, strong and familiar as in experiment 2 (Figs. 3B, left, S1C, and S2C). It also elicited anxiety (Fig. 3B, middle, 10% DMDS, $t(33) = 6.90$, $P \leq .01$), and significantly increased sAA secretion (Fig. 3B, right, 10% DMDS, $Z = 3.15$, $P \leq .01$)

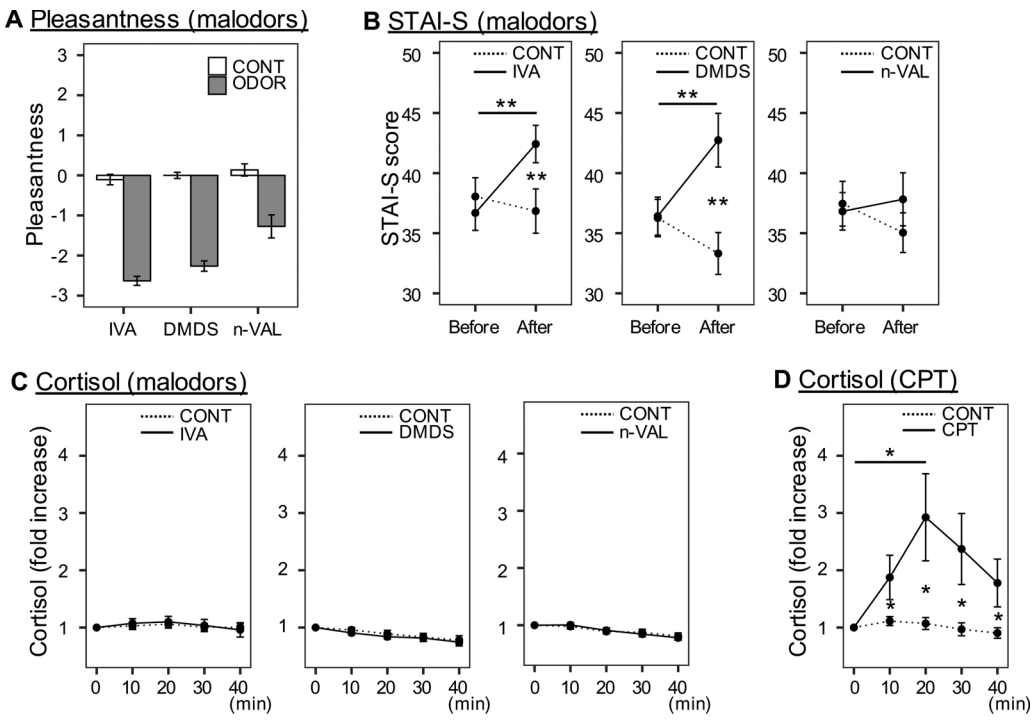

Fig. 2. Effects of malodors on salivary cortisol secretions (experiment 1). (A) Rated pleasantness of control (white bars) and odor (gray bars) samples. Pleasantness, -3 = very unpleasant, 3 = very pleasant. (B) Anxiety ratings measured using STAI-S before and after odor exposure obtained in odor (solid lines) and control (dashed lines) conditions. Possible STAI-S score ranges from 20 to 80, where a larger score means greater anxiety. (C) Time course of fold increase in salivary cortisol obtained for odors, and (D) for cold pressor test (CPT). Fold increase of cortisol was calculated as each of the post exposure (10 to 40 min) concentrations divided by a pre-exposure concentration. Values represent mean and standard errors. $^{**}P \leq .01$, $^{*}P \leq .05$. Abbreviations of odors are shown in Supplementary Table 1.

as the results of experiment 2 (Fig. 3A, middle and right). DMDS at lower concentrations was rated as less intense and less unpleasant (Figs. S1C and 3B left) and did not induce significant change in sAA secretion (1% DMDS, $Z = 1.78$, $P = .30$; 0.1% DMDS, $Z = 1.10$, $P = 1$; 0.01% DMDS, $Z = 1.92$, $P = .22$) (Fig. 3B, right).

These results also suggest that exposure to highly unpleasant odors increases secretion of sAA.

2.4. Experiment 4: effects of a pleasant odor on the secretion of sAA (perfume)

In experiments 2 and 3, the perceived intensity of odors that significantly increased sAA secretion was relatively higher compared to odors that did not. To exclude a possibility that odors of high-perceived intensity elicit SNS responses regardless of the pleasantness, we examined sAA secretion to a pleasant odor of high-perceived intensity.

2.4.1. Methods

Perfume (PER; Supplementary Table 1) was presented to a separate group of participants (n = 30; Supplementary Table 3). Otherwise, methods were the same as in experiment 2.

Details are provided in the Supplementary Materials and Methods.

2.4.2. Results

Intensity of PER was similar with IVA (Fig. S1B, D), and it was rated as pleasant and familiar (Figs. 3C, left, and S2D). Anxiety was significantly lower in PER than odorless control condition (PER, $t(29) = -2.59$, $P \leq .05$) (Fig. 3C, middle). No significant increase in sAA was observed in PER compared to the control condition (sAA, $Z = .61$, $P = .54$) (Fig. 3C, right).

This result suggests that secretion of sAA is not increased by odors with high perceived intensity when the odor is pleasant.

2.5. Experiment 5: effects of verbal information on the secretion of sAA

In order to separate the effect of an odor's unpleasantness from that of odor substance, we examined sAA secretions for the same odor substance, while manipulating its pleasantness by attaching different verbal information to the odor. To exclude the possibility that the observed effect is solely due to the verbal information, we also conducted a control experiment, where negative verbal information was attached to an odorless sample.

2.5.1. Methods

We used DMDS (Supplementary Table 1), the odorant that did not significantly increase sAA secretions in experiment 3, at a concentration of 0.1%. A between-participant design was used, where participants in one group received the odor with neutral information, "odor in bottle A" (n = 30), and participants in the other group received the odor with negative information, "a component of halitosis odors in bottle A" (n = 32; Supplementary Table 3). Both groups also underwent a control session, where empty bottles were presented with neutral information.

In addition, we conducted a control experiment using a separate group of participants (n = 30; Supplementary Table 3), where the effect of the negative verbal information (halitosis) was examined using an odorless sample (Supplementary Table 3).

The timeline of the experimental session (Fig. 1B), the measures taken, and the analysis method were the same as in experiment 2, except that participants were additionally asked to describe any objects that the odors brought to mind.

Details are provided in the Supplementary Materials and Methods.

2.5.2. Results

The perceived pleasantness and intensity of DMDS, and anxiety scores obtained after the exposure to DMDS were significantly modulated by the negative information associated with the odor (Figs. 4A left, middle, and S1E). Two-way mixed ANOVA revealed significant interactions between odor (control / DMDS) and group (neutral-information group / negative-information group) for pleasantness ($F_{1,60} = 23.34$, $P \leq .01$), intensity ($F_{1,60} = 9.78$, $P \leq .01$) and anxiety ($F_{1,60} = 9.23$, $P \leq .01$), but not for familiarity ($F_{1,60} = .03$, $P = 1$) (Fig. S2E). When the simple main effects of group were examined separately for control and DMDS, we found that DMDS was perceived as significantly more unpleasant and more intense (Pleasantness, $t(60) = -5.42$, $P \leq .01$; Intensity, $t(60) = 4.01$, $P \leq .01$), and exposure to DMDS increased anxiety significantly more (anxiety, $t(60) = 3.45$, $P \leq$

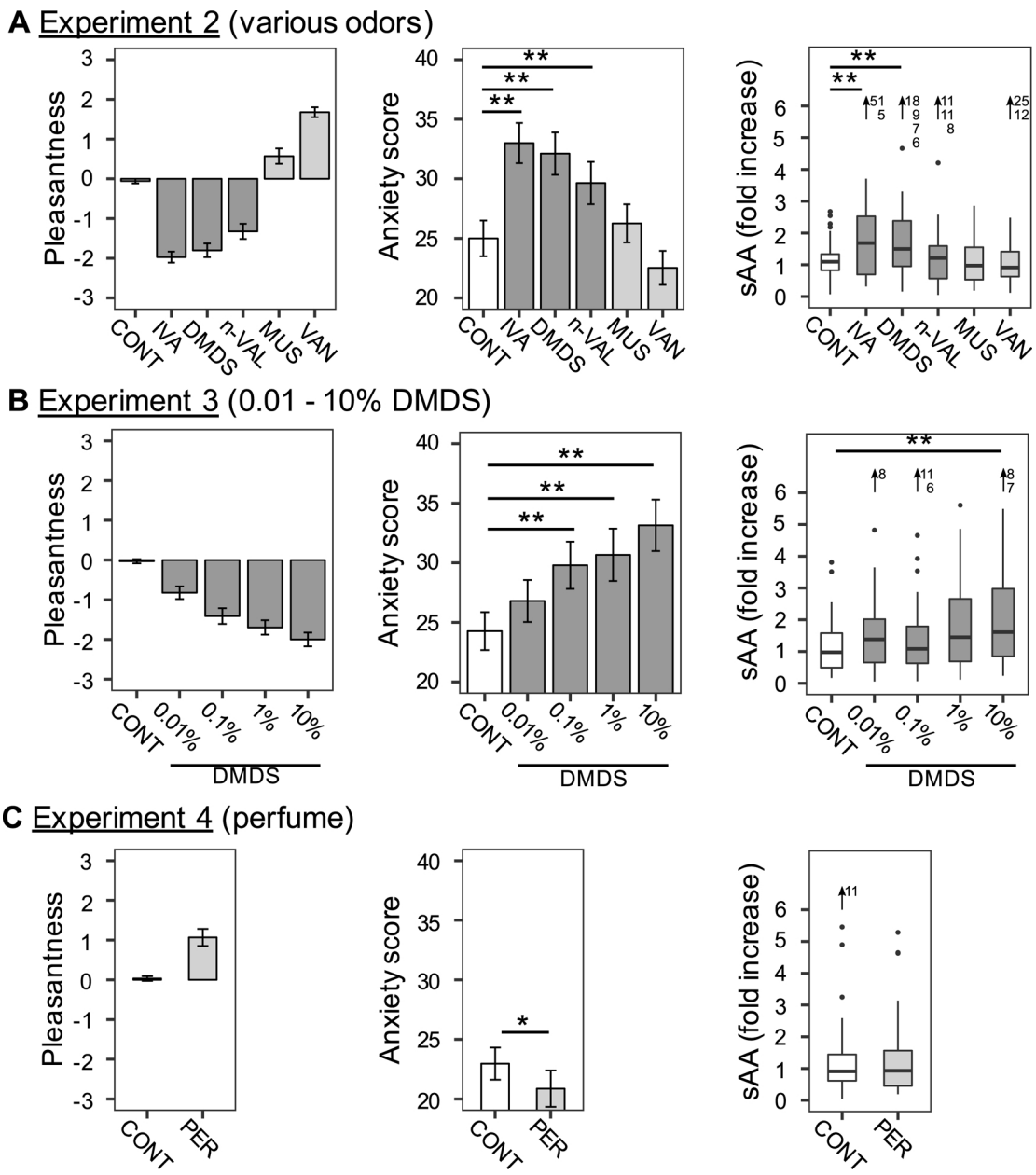

Fig. 3. Effects of various odors on sAA secretions (experiments 2–4).
Results of experiments on various odors (A), 0.01–10% DMDS (B), and perfume (C). From the left, bar graphs show rated pleasantness and anxiety scores, and box plots show fold increase of sAA secretion (post-exposure / pre-exposure). Possible anxiety score ranges from 0 to 60, where larger score denotes greater anxiety. Means and standard errors are presented in bar graphs, and medians (bands inside boxes), quartiles (boxes), 1.5 times the interquartile ranges (whiskers) and outliers (circles and scores beside arrows) are shown in box plots. White bars show control, dark gray bars show malodor and light gray bars show pleasant odor conditions. $^{**}P \leq .01$, $^{*}P \leq .05$. Scales used for pleasantness ratings are the same as Fig. 2. CONT, Control; sAA, salivary α-amylase. Abbreviations of odors are shown in Supplementary Table 1.

.01) for the negative-information group than for the neutral-information group.

Information also affected objects that the DMDS odor brought to mind (Fig. S3A). Significantly more participants thought of food in a neutral (83%) rather than negative-information group (47%) ($\chi^2 = 8.99$, $P \leq .01$), and significantly more participants thought of body-related objects in the negative (27%) than neutral-information group (0%) ($\chi^2 = 8.61$, $P \leq .01$).

Changes in sAA secretion upon odor exposure are shown in Fig. 4A, right. A significant increase of sAA secretion under the DMDS compared to the odorless control condition was observed only for the negative-information group ($Z = 2.23$, $P \leq .05$), but not for the neutral-information group ($Z = .52$, $P = 1$).

The results of the control experiment showed that an odorless sample (PG) with the negative information was rated as neutral in pleasantness (Fig. 4B left), and low in intensity and familiarity (Figs. S1F, S2F). Exposure to PG with the negative information did not induce significant increase of anxiety ($t(29) = 1.67$, $P = .11$) (Fig. 4B, middle) and sAA secretion ($Z = .63$, $P = .53$) (Fig. 4B, right) compared to the odorless control condition.

These results suggest that the levels of sAA secretion upon odor exposures are influenced by non-olfactory information attached to the odors, which influences perceived unpleasantness of the odors.

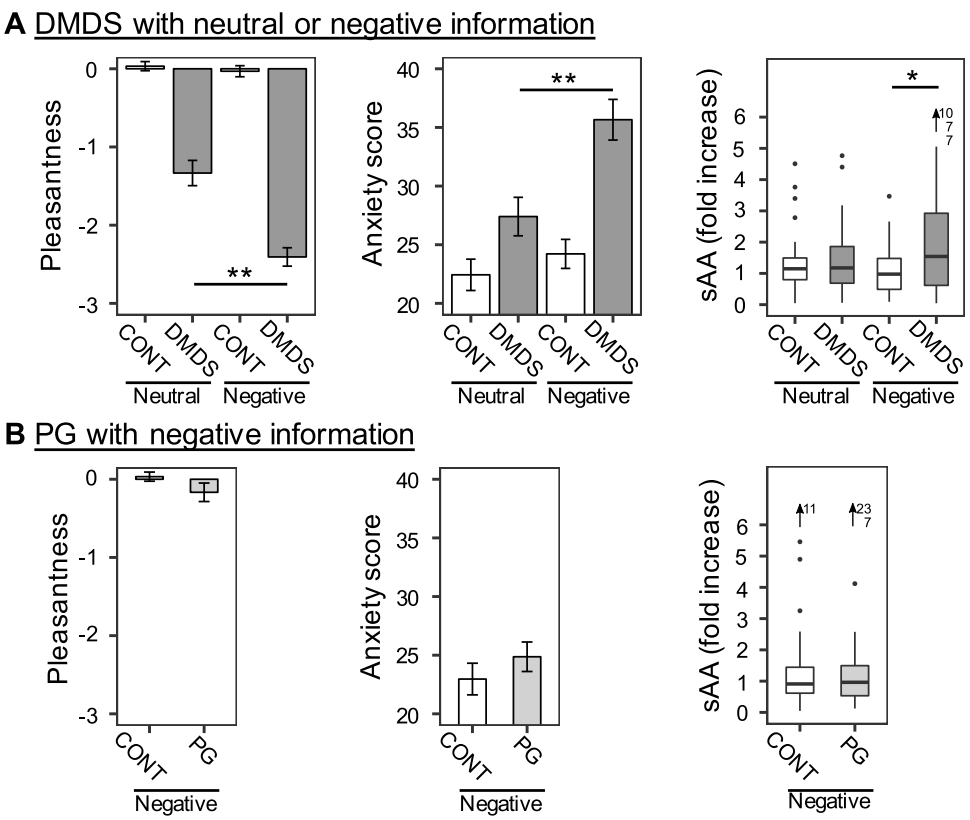

Fig. 4. The effects of verbal information on sAA secretions (experiment 5).
(A) Effects of verbal information on an odor (DMDS) sample. Results from neutral-information group are presented on the left, and negative-information group are presented on the right. (B) Effects of verbal information on an odorless (PG) sample obtained in the control experiment. The control experiment included only a negative-information group. Bar graphs and box plots represent descriptive statistics in the same manner as in Fig. 3. $^{**}P \leq .01$, $^{*}P \leq .05$. CONT, Control; sAA, salivary α-amylase. Abbreviations of odors are shown in Supplementary Table 1.

2.6. Experiment 6: effects of odor masking on the secretion of sAA

To separate the effect of an odor's unpleasantness from that of odor substance, the odor masking effect, a phenomenon during which an addition of odors suppresses the unpleasantness of the original odor, was used. In particular, we examined whether a change in pleasantness of a malodor caused by mixing with another odor influenced sAA secretion when the concentration of the malodor in the gas phase was the same.

2.6.1. Methods

We presented IVA mixed with VAN (IVA-VAN) or PG (IVA-PG; Supplementary Table 1) to a group of participants (n = 35; Supplementary Table 3). IVA-VAN was used, because VAN was found to change the perception of IVA in a pilot study. Concentrations of IVA in the headspace, assessed by GC-MS, were not significantly different between IVA-VAN and IVA-PG ($t(20) = 1.08$, $P = .30$). Timeline of the experimental session (Fig. 1B), the measures taken, and the analysis method were the same as in experiment 5.

Details are provided in the Supplementary Materials and Methods.

2.6.2. Results

Odor pleasantness largely differed between IVA-VAN and IVA-PG (Fig. 5left). A one-way ANOVA revealed a significant effect of odor on pleasantness, intensity, and familiarity (Pleasantness, $F_{2,68} = 44.47$, $P \leq .01$; Intensity, $F_{1.69,57.32} = 204.62$, $P \leq .01$; Familiarity, $F_{2,68} = 60.30$, $P \leq .01$). Follow-up *post hoc* comparisons showed that IVA-PG was significantly more unpleasant than IVA-VAN ($t(34) = -6.35$, $P \leq .01$), as well as control ($t(34) = -9.45$, $P \leq .01$). Intensity and familiarity were not significantly different between IVA-PG and IVA-VAN (Intensity, $t(34) = .93$, $P = .36$; Familiarity, $t(34) = -.94$, $P = .35$) (Figs. S1G, S2G).

The types of objects that participants thought of after exposure to odors also differed. While IVA-PG conjured images of objects such as natto (fermented soybeans), chocolate, sweat, and feet, it was mostly chocolate for IVA-VAN (Fig. S3B). The percentage of participants who thought of chocolate was significantly higher for IVA-VAN than for IVA-PG ($\chi^2 = 13.57$, $P \leq .01$).

The anxiety scores also significantly differed between odor conditions ($F_{2,68} = 15.39$, $P \leq .01$). *Post hoc* comparisons showed that participants were significantly more anxious after smelling IVA-PG than IVA-VAN ($t(34) = 3.57$, $P \leq .01$), or than control ($t(34) = 5.79$, $P \leq .01$) (Fig. 5 middle). While IVA-PG increased sAA secretion significantly more than odorless control condition, IVA-VAN did not (IVA-PG, $Z = 3.08$, $P \leq .01$; IVA-VAN, $Z = .98$, $P = .65$) (Fig. 5, right).

These results show that the sAA response to a malodor is affected by perceptual changes caused by the presence of another odor.

2.7. Correlation between the stress responses and odor ratings (Experiments 2–6)

To examine the effects of pleasantness on the SNS and emotional stress responses, we analyzed the relationship between sAA secretion, anxiety, and odor perception using the data obtained in experiments 2–6.

2.7.1. Methods

Following the previous studies that examined the relationships between odor perception and physiological responses (Alaoui-Ismaili et al., 1997; Bensafi et al., 2002a; 2002b; 2002c; Delplanque et al., 2008; Glass et al., 2014; He et al., 2014, 2016), we conducted an odor-wise correlation analysis. In brief, the median for sAA between participants, and the means of the subjective ratings (pleasantness, intensity, familiarity, and anxiety scores) between participants were calculated for each of the 21 odor conditions collected from five groups of participants (Supplementary Table 3). Since a preliminary analysis showed that participant group-identity did not have significant effects on stress responses (Supplementary Materials and Methods), data from five participants groups were pooled. The relationship between odor ratings and stress response was examined using the Pearson's correlation

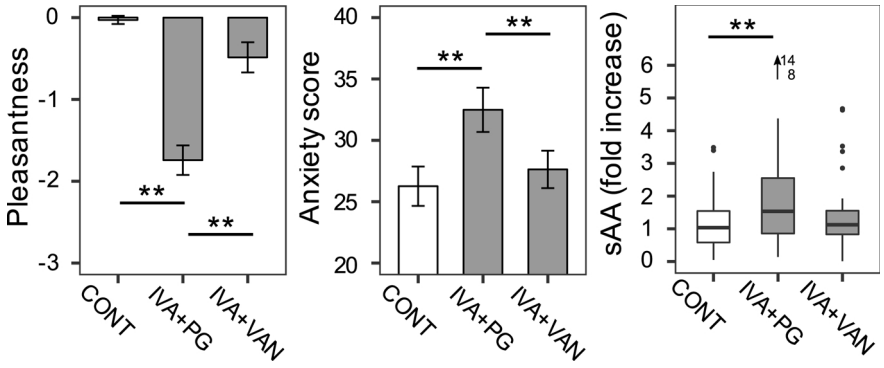

Fig. 5. The effects of odor masking on sAA secretions (experiment 6).
Results of experiments on mixtures of IVA and PG or VAN. Concentrations of IVA in the gas phase were the same for IVA + PG and IVA + VAN samples. Bar graphs and box plots represent descriptive statistics in the same manner as in Fig. 3. $^{**}P \leq .01$. Scales used for pleasantness and intensity ratings are the same as Fig. 3. CONT, Control; sAA, salivary α-amylase. Abbreviations of odors are shown in Supplementary Table 1.

analysis, and partial correlation analysis. For verification, we also conducted a multilevel analysis that included individual variability in the models, and found significant associations between an odor's unpleasantness and a stress response (Supplementary Tables 4 and 5).

Details are provided in the Supplementary Materials and Methods.

2.7.2. Results

Pearson's correlation analysis showed that stress responses of emotion (anxiety score) and SNS (sAA secretion) were strongly correlated ($r = .88$, $P \leq .01$) (Fig. S4A). Significant strong negative correlations were observed between odor pleasantness and both anxiety and sAA responses (Anxiety, $r = -.92$, $P \leq .01$; sAA, $r = -.86$, $P \leq .01$) (Fig. 6). There were weaker, but significant correlations between stress responses and perceived intensity (Anxiety, $r = .67$, $P \leq .01$; sAA, $r = .63$, $P \leq .05$) (Fig. S4B, C), while no significant correlations were observed between stress responses and familiarity (Anxiety, $r = .26$, $P = 1$; sAA, $r = .24$, $P = 1$) (Fig. S4D, E).

Since pleasantness, intensity, and familiarity ratings correlated with each other, we also calculated partial correlation between each of the odor ratings (e.g, pleasantness) and the stress responses, while controlling for the other two (e.g., intensity and familiarity). Significant correlations were observed only between pleasantness and the stress responses (Pleasantness-Anxiety, $pr = -.79$, $P \leq .01$; Pleasantness-sAA, $pr = -.63$, $P \leq .05$; Intensity-Anxiety, $pr = .16$, $P = 1$; Intensity-sAA, $pr = .15$, $P = 1$; Familiarity-Anxiety, $pr = .05$, $P = 1$; Familiarity-sAA, $pr = -.01$, $P = 1$).

These results suggest that subjective pleasantness has a significant relationship with stress responses observed as anxiety and sAA secretion.

3. Discussion

We examined stress responses elicited by malodors, and their association with perceived unpleasantness of the odors in this report. To this end, we presented malodors specified as offensive in the Offensive Odor Control Law of Japan. As expected, these odors were rated as unpleasant, and exposure to them increased self-reported anxiety, an emotional state associated with stress. Secretion of sAA, a marker of SNS, significantly increased while salivary cortisol, a marker of HPA activity, did not (Figs. 2,3). Further examination of sAA responses showed that inhalation of the same odorous substances induced SNS response depending on the level of unpleasantness (Figs. 4,5). Changes of sAA secretions and levels of odor-elicited anxiety were strongly correlated with perceived unpleasantness of odors (Fig. 6). These results demonstrate that malodors can induce stress response of emotion and SNS, and that these stress responses are relative and depend upon the perceived unpleasantness of odors.

3.1. Malodors elicit emotional and SNS-related stress responses

The first question we addressed was whether malodors elicit emotional and physiological stress responses. Our data show that anxiety and SNS activity increase upon exposure to malodors. Anxiety is characterized as emotional response to stressors, and increase of anxiety measured by STAI-S has been documented under various stressful conditions (Rossi and Pourtois, 2012). Therefore, significant increase in STAI-S scores (Fig. 2) and shortened STAI-S (Figs. 3–6) upon exposure to malodors indicate that stress-related emotional change is elicited by malodors. Increase in anxiety is also in line with previous field surveys that showed negative mood, including anxiety, among the residents of malodourous environment (Horton et al., 2009; Wing et al., 2013).

While previous studies have examined how SNS responds to odors, none of them, to our knowledge, focused on stress, and examined the association between the SNS activity and other stress-related measures (Alaoui-Ismaili et al., 1997; Bensafi et al., 2002a, b; Delplanque et al., 2008, 2009; Glass et al., 2014; He et al., 2014, 2016; Pichon et al., 2015). Our study is the first to show SNS activation in the context of stress-related emotional responses elicited by malodors. In addition to the absence of stress measures, due to the differences in the intent of the research, the experimental designs of the previous studies differed from ours. First, durations of odor presentation in the previous studies were brief, mostly less than several seconds. Second, levels of unpleasantness of odors examined in the previous study were not necessarily high. Thus, comparison of our results with previous reports needs to be performed with caution. Nevertheless, the majority of these studies revealed increases in SNS markers, such as cardiovascular (Alaoui-Ismaili et al., 1997; Bensafi et al., 2002a, b; Delplanque et al., 2009; He et al., 2014; Pichon et al., 2015) and skin conductance responses (Alaoui-Ismaili et al., 1997; Delplanque et al., 2008; Glass et al., 2014; He et al., 2016) upon exposure to unpleasant odors, and our results were consistent with these previous studies.

3.2. Perceived unpleasantness affects the stress response

The second question we researched was whether perceived unpleasantness of odor itself can elicit stress responses. An affirmative answer to this question was supported from two perspectives. First, both levels of anxiety and sAA secretion significantly and strongly correlated with a subjective pleasantness of odors (Fig. 6). Second, by using context effects, stress responses to the inhalation of identical odorous substances were shown to be modulated together with the perceived pleasantness of the odor. A significant increase of sAA secretion was observed for 0.1% DMDS (weak garlic-like odor) only when it was presented with a negative information (a component of halitosis odors), but not with a neutral information (Fig. 4). Inhalation of IVA (feet-like odor) mixed with PG (odorless) significantly increased sAA, but this increase was not elicited when IVA was mixed with VAN (vanilla-like odor), even though the concentration of IVA in the headspace was the same (Fig. 5). In both cases, perceived pleasantness and odor-induced anxiety were also modulated by the context: the DMDS with negative information and IVA with PG were rated more unpleasant and induced higher levels of anxiety compared to DMDS with neutral

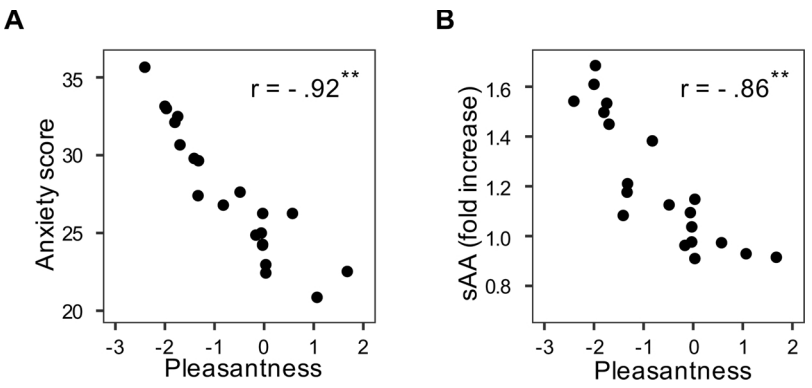

Fig. 6. The relationship between rated pleasantness and stress responses.
Each dot represents the inter-participant summary of an odor condition. Data for all the 21 odor conditions examined in sAA experiments are presented. Pleasantness (possible range -3 to 3) and anxiety (possible range 0–60) scores are inter-participant means, and sAA secretion changes (post-exposure/pre-exposure) are inter-participant medians. *r*, Pearson's correlation coefficients. $^{**}P \leq .01$. Scatter plots showing the relationship between odor intensity, familiarity and stress responses are presented in Fig. S4. sAA, salivary α-amylase.

information and IVA with VAN, respectively. To our knowledge, this is the first study to report the odor masking effect on a stress response to odors. On the other hand, there is a study that found an increase in SNS activity, measured as the skin conductance response to odors when the odors were presented with negative verbal information (Djordjevic, et al., 2008). Our result from the verbal information experiment was in line with their result.

It is possible that odor-induced stress responses are substance dependent, rather than perception dependent. For example, odorous substances could cause irritation, as most of the odorants stimulate trigeminal nerves in addition to olfactory nerves (Hummel and Livermore, 2002). In addition, odorous substances may enter the blood stream via nasal or lung mucosa and exert pharmacological effects (Moss and Oliver, 2012). Although such substance dependent effects may exist, the current results showed that unpleasantness itself can cause a stress response. Such a finding is important, as in real life situations, context dependent changes in pleasantness of odors seem likely to occur. The influence of cognitive factors, including labeling, on the perceived pleasantness of odors is well known (Djordjevic et al., 2008; Herz, 2003; Herz and von Clef, 2001; Stevenson, 2011). Masking unpleasant odors by using pleasant odors, such as fragrances, are a common practice. Our results suggest that changes in stress responses can accompany such modulation of perceived unpleasantness caused by the context in which the odors are inhaled.

3.3. Relationship between the secretion of sAA and activities of autonomic nervous system

Although it is indisputable that the SNS influences sAA secretion, and sAA is often used as an SNS marker (Nater and Rohleder, 2009; Segal, 2016), activity of the parasympathetic nervous system (PNS) can affect sAA (Bosch et al., 2011; Nagy et al., 2015; Proctor and Carpenter, 2007). There are two major factors to consider: 1) the fluid secretion into saliva is controlled by the PNS, and therefore, the sAA concentration in saliva is influenced by the PNS; 2) The sAA secretion itself is influenced by the PNS. Regarding the former factor, we used sAA secretion (U/min), instead of sAA concentration (U/ml) to avoid the influence of fluid secretion, and thus the influence of the PNS, as recommended previously (see the Supplementary Materials and Methods) (Bosch et al., 2011). However, the possible involvement of the second factor still remains. The impact of the PNS on sAA secretion is not yet clear. In animal studies, concurrent stimulation of the PNS and the SNS is shown to increase sAA secretion compared to the stimulation of the SNS alone (Proctor and Carpenter, 2007). Synergistic effect of the PNS and SNS in sAA secretion is also suggested in humans (Nagy et al., 2015). Therefore, the current observations of sAA increase with malodors may reflect both SNS and PNS activation. Considering that the responses of SNS markers to stressors are not uniform (Grassi et al., 2008; Schumacher et al., 2013), measuring the activity of the SNS and PNS using multiple markers would be useful for a comprehensive understanding of odor-elicited stress responses.

3.4. Malodors do not affect the secretion of salivary cortisol

Salivary cortisol levels did not change in our study (Fig. 2C). This lack of cortisol response was somewhat surprising considering that HPA had been shown to respond to a variety of stressors, including unpleasant visual (Codispoti et al., 2003) and auditory stimuli (Wagner et al., 2010). The null finding with malodor was unlikely to be a simple technical error, considering that we could successfully detect significant HPA response to the Cold Pressor Test, one of the standard stress tasks known to induce an HPA response (Fig. 2D). There was also a possibility that odor adaptation occurred during the 40 min of odor exposure. Nonetheless, participants rated high unpleasantness and felt high anxiety after malodor exposures, so unpleasant odors must have affected participants.

Previous studies have shown that HPA does not necessarily respond to all types of stressors. A meta-analysis of 208 laboratory studies on acute psychological stress tasks suggested that HPA activity was elicited when the task was uncontrollable, and/or involved social-evaluative threat (Dickerson and Kemeny, 2004). Some studies did not find an HPA response but found SNS responses to the same stressors, and suggested that sensitivity of HPA and SNS to stressors differed across type of stressors (Frankenhaeuser, 1982; Maruyama et al., 2012; Skosnik et al., 2000; van Stegeren et al., 2008; Wetherell et al., 2006; Wiemers et al., 2013). In the current study, we did not examine the HPA and SNS in the same experiment. Therefore, the results cannot be compared directly. However, common malodors (IVA, DMDS and n-VAL) were used for both cortisol and sAA experiments at the same concentrations. Moreover, the duration of odor exposure in the cortisol experiment was longer than in the sAA experiment. Considering this, our results may indicate that for the type of stress elicited by malodors, the HPA is less sensitive than SNS.

It should be noted, however, that our result does not preclude a possibility that malodors are able to elicit an HPA response. The current study examined acute stress responses, where participants knew that they could escape from the odor in 40 min or so. Chronic exposure to malodors, or exposure to odors under more uncontrollable situations, which could occur in real life, may involve an HPA response. Moreover, in rodents, it is known that odors associated with fear, both innate and learned, induce an HPA response (Isosaka et al., 2015). Thus, examination of HPA responses under a wider range of exposure condition and odorants might be warranted.

3.5. sAA as an indicator of odor impact

An important future direction of our study is the further characterization of the sAA response to malodors, in order to use it as an indicator of odor impact. Quantification of the impact of odors arising from their sensory quality is challenging. Odors are often regulated by

measuring and controlling the ambient air concentrations of malodorous substances (Brancher et al., 2017; Hayes et al., 2014). However, environmental malodors are often complex mixtures of odorous substances. In such a case, concentration of an individual chemical does not always correspond to the impact of an odor as a whole, as perceived qualities of odors might change in mixtures of multiple components. To evaluate such complex sensory impacts, subjective reports such as sensory evaluation of odors, and/or counting numbers of complaints from residents has been employed. As shown in the current study, sAA can be a sensitive biomarker for perceived unpleasantness of odors and odor-elicited stress. Saliva collection is non-invasive and sAA is easy to assess. Therefore, sAA might be a useful objective indicator of the impact of malodors on humans, supplementing currently employed assessment methods.

4. Conclusion

In conclusion, our study showed that unpleasantness of odor elicits stress responses observed as changes in emotion and SNS-related physiological activities. We also showed that the property of an odor as a stressor depends, at least partly, on how the odor is perceived. Finally, our results show that sAA is a sensitive marker of the odor-elicited stress response. In this study, we focused on the stress responses to odors. However, humans are often exposed to malodors with various other stressors (e.g. waste treatment, nursing, and so on). Hence, examining the synergistic effect of malodors on stress responses to other concurrent stressors would be also important. The current study, and further studies using sAA and related markers, will contribute to the understanding of effects of how malodors affect humans, as well as the nature of human stress responses.

Author contributions

Conceived and designed the experiments: YH MS MO KT. Performed the experiments: YH MO. Analyzed the data: YH MS MO KT. Contributed reagents/ materials/ analysis tools: YH MO KT. Wrote the paper: YH MO. All authors have approved the final article.

Conflict of interest

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.03.018.

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